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THE EFFECT OF EARLY SPRING FLOODING ON CERTAIN FORAGE CROPS[1]

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Since the early settlement of Western Canada many spring flooding projects have been developed, the majority of which are devoted principally to the production of perennial forage crops. This type of forage crop production is vitally important to the livestock industry on the short-grass plains in order to insure feed supplies during dry years. Some of these projects are so constructed that spring run-off water may be held on the land for any desired length of time and then allowed to drain. In other instances drainage is not possible and the spring run-off water remains until lost through seepage and evaporation. This is particularly the case in regard to sloughs of which more and more are being seeded down to perennial crops due to the general difficulties of annual crop production.

It is evident that, in order to survive, any forage crop seeded under such conditions would have to be able to tolerate the longest period of spring flooding which would occur. Because of the demand for information on suitable crops to grow on spring flooding projects and to use for seeding down sloughs, experiments were started in 1939[4] to obtain information on the approximate upper limit of tolerance to early spring flooding of several commonly grown forage crops. In so far as the authors are aware, no experimental data have been published on this subject and consequently no literature is cited.

METHOD

The experiments were started at the Val Marie Irrigation Project in 1939 and continued until 1942. Similar experiments were conducted at the Eastend Irrigation Project in 1940, 1941 and 1942. At Val Marie a saline very heavy clay soil was used and at Eastend a clay loam which was slightly saline. Both irrigation projects are located in south-western Saskatchewan.

The crops tested were seeded between two border ditches in parallel strips from 8 to 10 feet wide depending on the type of drill used. During the first year the stands were irrigated as required and in the fall of the first year dikes were constructed at right angles to the strips of crops.

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This arrangement provided a number of basins, each of which could be filled with irrigation water and drained independently of the adjacent basin. The dikes were placed 40 feet apart and the border ditches were 90 feet apart so that each basin measured 40 by 90 feet and contained a plot 40 feet long and 8 or 10 feet wide of each crop to be tested.

In the spring of the second year after seeding and as soon as irrigation water was available, each basin was flooded to a depth of 12 to 18 inches. The water was subsequently drained from each basin at regular intervals ranging from 3 to 49 days after flooding. Flooding was done before growth started, usually in early April, and reflooding from time to time was necessary in order to maintain the water at the desired depth as losses resulted from seepage and evaporation.

All treatments were carried out in duplicate and data were obtained by taking observational notes on the percentage of permanent injury caused to a particular crop by a given period of flooding. These notes were recorded in early July.

The following crops were included in the experiments:

Biennial white sweet clover	(*Melilotus alba* Desv.)
Grimm alfalfa	(*Medicago media* L.)
Fairway crested wheatgrass	(*Agropyron cristatum* (L) Gaertn.)
Parkland brome grass	(*Bromus inermis* Leyss.)
Grazier slender wheatgrass	(*Agropyron pauciflorum* (Schwein) Hitchc.)
Meadow fescue	(*Festuca elatior* L.)
Commercial timothy	(*Phleum pratense* L.)
Reed canary grass	(*Phalaris arundinacea* L.)

RESULTS AND DISCUSSION

Data from the experiments are presented in Tables 1 to 6.

The results obtained from these experiments indicate that among the various forage crops there are wide differences in ability to withstand early spring flooding and furthermore there appears to be a definite upper limit of tolerance for each particular crop. This upper limit, while variable, is essentially similar from year to year and from station to station. Climatic conditions at Eastend are somewhat different to those at Val Marie. The respective elevations are 2,995 and 2,602 feet above sea level; consequently, the frost-free period is shorter and there are cooler conditions in the spring

TABLE 1.—ESTIMATED PERCENTAGE INJURY TO SWEET CLOVER

Number of days flooded	1939	1940	1941	1942	
	Val Marie	Eastend	Val Marie	Val Marie	Eastend
7	0	0	0	0	0
14	5	0	95	65	50
17	65	95	100	100	100
21	100	100	100	100	100

TABLE 2.—ESTIMATED PERCENTAGE INJURY TO ALFALFA

Number of days flooded	1939	1940		1941		1942	
	Val Marie	Val Marie	Eastend	Val Marie	Eastend	Val Marie	Eastend
10	0	0	0	0	0	0	0
14	5	95	0	95	0	5	0
17	25	100	95	100	50	80	50
21	100	100	100	100	100	100	60
28	100	100	100	100	100	100	100

TABLE 3.—ESTIMATED PERCENTAGE INJURY TO CRESTED WHEATGRASS

Number of days flooded	1939	1940		1941	1942	
	Val Marie	Val Marie	Eastend	Val Marie	Val Marie	Eastend
10	0	0	0	0	0	0
14	0	60	25	55	0	10
17	40	85	50	—	0	50
21	100	100	100	100	5	100
28	—	100	100	100	90	100

TABLE 4.—ESTIMATED PERCENTAGE INJURY TO BROME GRASS

Number of days flooded	1939	1940		1941	1942	
	Val Marie*	Val Marie†	Eastend‡	Val Marie	Val Marie	Eastend§
21	0	0	0	0	0	0
24	—	—	0	10	0	0
28	—	50	0	65	0	0
31	—	—	—	85	0	30
35	—	75	—	100	30	50
49	—	—	—	—	100	—

* 21 days longest period flooded in 1939.
† 35 days longest period flooded in 1940.
‡ 28 days longest period flooded in 1940.
§ 35 days longest period flooded in 1942.

TABLE 5.—ESTIMATED PERCENTAGE INJURY TO SLENDER WHEATGRASS

Number of days flooded	1941	1942	
	Val Marie*	Val Marie	Eastend†
28	0	0	0
31	0	—	10
35	5	40	45
42	—	—	—
49	—	100	—

* 35 days longest period flooded in 1941.
† 35 days longest period flooded in 1942.

TABLE 6.—ESTIMATED PERCENTAGE INJURY TO MEADOW FESCUE

Number of days flooded	1941	1942	
	Val Marie*	Val Marie	Eastend†
24	10	0	0
28	45	0	25
31	80	0	30
35	100	0	45
49	—	0	—

* 35 days longest period flooded in 1941.
† 35 days longest period flooded in 1942.

at Eastend. This may account for the fact that the various crops tested there appeared to withstand slightly longer periods of flooding at this Station. This is also probably due to some extent to the fact that difficulty was usually experienced in obtaining proper flooding at the beginning of the experiments at Eastend. The soil here has good sub-surface drainage and the initial floodings each year seeped away necessitating continuous reflooding until a satisfactory level could be maintained on the plots. Such was not the case at Val Marie as the soil is relatively impervious and here the first flooding was usually satisfactory, reflooding only being necessary to make up for evaporation and slight seepage losses.

It was observed that the nature of the weather in the spring of the year influenced considerably the length of time some crops may be flooded. The average monthly temperatures for April, May and June in 1941 and 1942 at Swift Current, the closest Station for which records are available, are shown in Table 7. These data indicate the differences between the two seasons.

A cool spring appeared to increase the tolerance of crested wheatgrass, brome, meadow fescue, alfalfa and sweet clover, whereas a warm spring had the opposite effect. For example, alfalfa sustained 95% damage at Val Marie in 1941, a warm spring year, from 14 days flooding, and 50% at Eastend, from 17 days flooding. In 1942, a cool spring, alfalfa at Val Marie only suffered 5% damage in 14 days. In that year at Eastend alfalfa was damaged to the same extent in 17 days as it had been in 1941 but was not completely killed out until it had been flooded a total of 28 days whereas in 1941 it was killed out in 21 days. Similar situations in these two years with respect to other crops are apparent.

There is some indication that cool spring weather exerts an opposite effect on slender wheatgrass. While only a small amount of damage resulted from 35 days flooding at Val Marie in 1941, there was 40% injury sustained in 1942 when cool conditions prevailed.

Timothy and reed canary grass were included in the experiments at Val Marie in 1941 and 1942 and at Eastend in 1942. These two grasses were not injured by 35 and 49 days flooding at Val Marie or by 35 days at Eastend. These two crops were the most resistant of those tested and appear to be most suitable for locations where extended periods of flooding occur. Table 8 indicates the range in days that the various crops may be spring flooded without causing excessive permanent injury as determined by the experiments reported on.

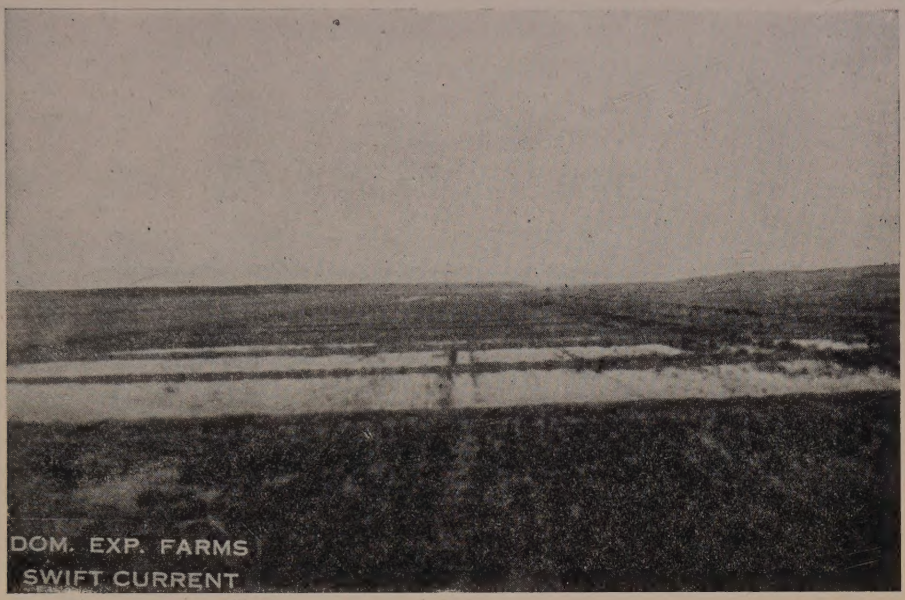

FIGURE 1. General view of the experiment at Val Marie in 1942, showing basins filled with water in the early spring.

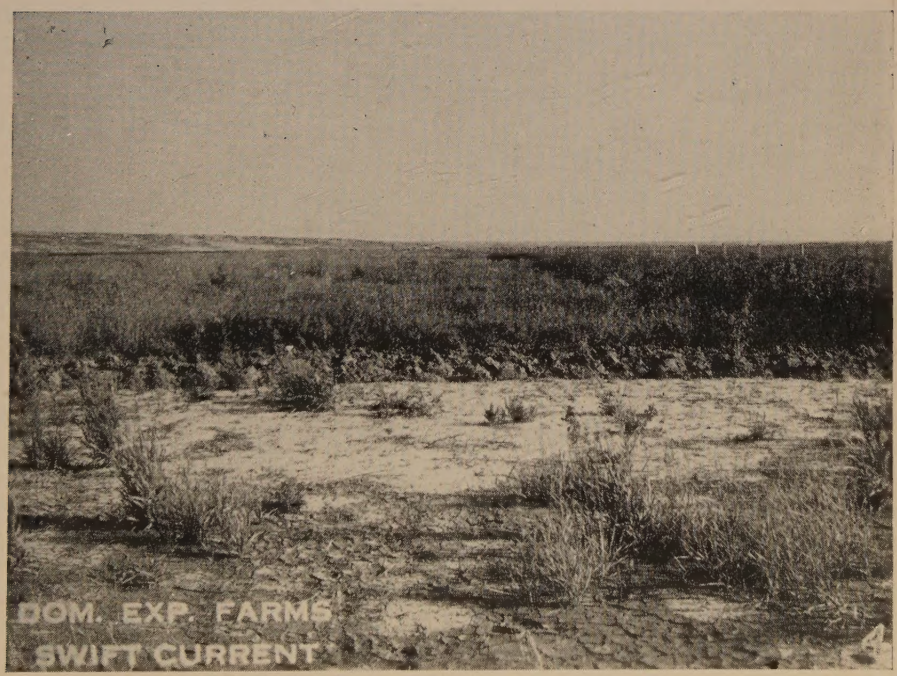

FIGURE 2. Alfalfa spring flooded for 3 days (background) and 21 days (foreground).

FIGURE 3. Brome grass flooded for 3 days (background) and 21 days (foreground).

FIGURE 4. Sweet clover flooded for 3 days (background) and 21 days (foreground).

TABLE 7.—AVERAGE MONTHLY TEMPERATURES AT SWIFT CURRENT IN 1941 AND 1942

Month	1941	1942
April	43.0	43.5
May	53.6	48.4
June	62.5	56.6

TABLE 8.—RANGE IN DAYS WHICH CERTAIN FORAGE CROPS MAY BE SPRING FLOODED WITHOUT CAUSING EXCESSIVE PERMANENT INJURY

Crop	Number of days	Crop	Number of days
Sweet clover	9–12	Slender wheatgrass	31–35
Alfalfa	10–14	Meadow fescue	24–35
Crested wheatgrass	10–17	Timothy	49*
Brome	24–28	Reed canary grass	49*

* Forty-nine days longest period tested. These two crops apparently will stand longer periods, particularly reed canary grass.

Sweet clover, alfalfa and crested wheatgrass may be classed as crops tolerant of shorter spring flooding periods, brome, slender wheatgrass and meadow fescue of relatively long periods, and timothy and reed canary grass of extended periods of flooding. Under practical irrigation conditions, this general relationship holds true during mid-season growing conditions as well and it has been observed that crops such as alfalfa are particularly sensitive to over irrigation or poor drainage of irrigation water whereas brome, slender wheat, meadow fescue, timothy and reed canary grass are not visibly affected.

The experimental results furnish a basis for advising farmers and ranchers as to the forage crop they should grow under their particular conditions, providing information is available on the approximate length of time their land is flooded in the spring. Suitable mixtures may also be worked out from the data obtained.

SUMMARY

As a result of the need for information on forage crops to grown under spring flooding conditions, experiments were conducted at the Val Marie and Eastend Irrigation Projects from 1939 to 1942 to determine the length of time which certain commonly grown forage crops may be covered with water without causing excessive permanent injury. Sweet clover, alfalfa and crested wheatgrass were able to withstand only short periods of flooding while brome, slender wheatgrass and meadow fescue were able to stand fairly long periods. Timothy and reed canary grass stood the most flooding of the crops tested.

PRELIMINARY EXPERIMENTS WITH BENZENE HEXACHLORIDE (666) AS AN INSECTICIDE[1]

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The insecticidal properties of benzene hexachloride, more precisely 1,2,3,4,5,6-hexachlorocyclohexane, $C_6H_6Cl_6$, and now commonly referred to as 666, appear to have been first discovered in France, where a patent for its preparation was issued as early as 1941, according to Bourne (1). It has been sold there for some years under the trade name of "Aphtiria," and was used in large quantities during the war by the French Government. In 1942 workers in England independently discovered its toxicity to insects, and it was quickly developed and used in quantity as an emergency insecticide, particularly against flea beetles. The history of its early development in England, for some time kept under military secrecy, has recently been published by Slade (2), who also gives an outline of its chemistry.

According to Slade, crude 666 is largely a mixture of the four known stereo-isomers of hexachlorocyclohexane, designated alpha, beta, gamma and delta, of which only the gamma isomer has appreciable insecticidal properties. Slade has suggested that the gamma isomer be known as *gammexane.* Crude 666, which is a pale brownish solid with a very pronounced, penetrating odour, contains 10 to 12% gamma isomer. The pure gamma isomer is a colourless crystalline solid, practically odourless, with a melting point of 112.5° C., insoluble in water but dissolving in many organic solvents. Because of difficulty in separating the isomers it is unlikely that the pure gamma isomer will be produced commercially, but so-called refined 666 containing up to 40% gamma isomer has been prepared.

Because of the similarity in solubility and other physical properties, 666 can in general be processed in the same manner as DDT. Like the latter, undiluted 666 is not suitable for most insecticidal uses because the slightly waxy consistency of the crude or refined material makes it very difficult to grind undiluted to the required degree of fineness. It is also difficult to wet. Dusts of 666 can be prepared by grinding with an inert powder such as talc, clay or pyrophyllite, and wettable spray powders in the same manner by adding a dispersing agent. It can also be dissolved in appropriate solvents and used as a solution, or the latter can be emulsified in water.

This paper presents the results of exploratory tests with small samples of 666 which were received at Vineland Station in 1944 and 1945.

MATERIALS AND FORMULATIONS

The materials tested at this laboratory to date have been the following, all except the last two being obtained from England[5]: (a) Pure gamma isomer; a five-gram sample; (b) crude 666, containing 10 to 12% gamma isomer; (c) refined 666, containing 30 to 40% gamma isomer; (d) a finely

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ground mixture of 20 parts crude 666 and 80 parts gypsum; (e) a miscible oil solution containing 17% crude 666; (f) a wettable spray powder containing 50% of a partially refined 666, the gamma content of the latter being approximately 22%. This sample was received from the United States[6] and will be referred to as American 666; (g) a dust containing 5% of American 666 (22% gamma). The gamma content in all cases is that given by the manufacturers. The methods used in determining these values are not known to the writers.

Most of the experiments were conducted with a standard wettable spray powder prepared in the laboratory by grinding for 17 hours in a ball mill the following: 666 (either pure gamma isomer, crude 666 or refined 666) 10%, Orvus (wetting agent containing 32% sodium lauryl sulphate) 3%, and pyrophyllite 87%. A similar formulation containing commercial DDT was used for comparative purposes. *Unless otherwise stated the10-3-87 formula was used and the Orvus concentration in the dilute spray adjusted to ½ lb. per 100 gallons.* Other formulae for dusts and sprays are described in the text.

The method of stating the composition of the spray or dust is as follows: in the tables the amounts in pounds per 100 gallons refer to the actual amount of crude 666, refined 666 or DDT as the case may be, and *not* to the amount of the complete formulation. In addition the approximate gamma isomer content in pounds per 100 gallons of spray is given in some cases. The latter is an average figure derived from the range stated by the manufacturer, viz., 11% for crude 666 and 35% for refined 666.

Test Insects and Methods

Experiments were conducted with 14 species of insects and 2 mites, some of which were chosen not for their economic importance but because they lent themselves readily to laboratory testing. As the work progressed it became evident that 666 had fumigant as well as contact and stomach poisoning action and consequently special techniques had to be devised.

Sprays were applied in all cases with a De Vilbiss paint sprayer, usually with the spray gun mounted on a rigid stand and the spray material mechanically agitated.

Codling Moth (*Carpocapsa pomonella* (L.)) and Oriental Fruit Moth (*Grapholitha molesta* (Busck.))

Toxicity of 666 to Newly Hatched Codling Moth Larvae

Wealthy apples were picked before maturity in 1944 and kept in cold storage until required in the early spring of 1945, when they were washed in 2% hydrochloric acid to remove old spray residue. After the calyx and stem ends were waxed, the fruits were sprayed individually, being revolved in the spray cone of a paint atomizer for 12 seconds in a machine specially constructed for this work. Codling moth eggs were obtained on waxed paper from moths reared from field-collected larvae. As soon as the spray had dried on the fruit, 10 eggs hatching within the next 24 hours

[5] Imperial Chemical Industries Ltd., Liverpool, England.
[6] Niagara Sprayer and Chemical Co., Inc., Middleport, N.Y.

were pinned on each apple and incubated at 77° F. and 75 to 85% relative humidity. Ten apples were used for each individual test. Both stings and entries were recorded three days later but the number of stings was so erratic that they have not been further considered. The entries in an unsprayed check (10 fruits—approximately 100 larvae), run along with the sprayed fruit in each day's test, were employed in calculating the percentage efficiency of the sprays according to Abbott's formula (J. Econ. Ent. 18 : 265-267).

For comparative purposes, both pure gamma 666 and relatively pure DDT (96 to 99% para-para isomer) were used in the form of the 10-3-87 grind with Orvus and pyrophyllite already described. Crude 666 was not employed in this form because the high concentration of the mixture required would contain too great a proportion of wetter. Lignin pitch was therefore used as a dispersing agent in grinds containing 10 parts crude 666, lignin pitch 3 parts, and pyrophyllite 87 parts; or 20 parts crude 666, lignin pitch 3 parts, and pyrophyllite 77 parts by weight. The preparation containing 20% crude 666 in gypsum was also tested. No wetter was employed in any of the tests beyond that already present in the dry formulation.

The results of the tests, shown in Table 1, indicated that pure gamma 666 was distinctly more toxic to codling moth larvae than DDT. The small sample of pure gamma 666 available prevented an extensive series of tests, but the data obtained suggested that its toxicity to codling moth larvae was in the neighbourhood of one and one-half times that of the para-para isomer of DDT.

TABLE 1.—RELATIVE TOXICITY OF 666 AND DDT TO NEWLY HATCHED CODLING MOTH LARVAE

Materials	Lb. per 100 gal.	Approx. gamma isomer content; lb. per 100 gal.	No. of replicates	Percentage efficiency (Abbott)
DDT (10-3-87 DDT-Orvus-pyrophyllite)	0.25	—	3	60.1
DDT (as above)	0.5	—	4	80.0
Pure gamma 666 (10-3-87 gamma isomer-Orvus-pyrophyllite)	0.25	0.25	3	73.6
Pure gamma 666 (as above)	0.5	0.5	3	93.3
Crude 666 (10-3-87, 666-lignin pitch-pyrophyllite)	2.5	0.275	4	58.4
Crude 666 (20-80, 666-gypsum)	5.0	0.55	4	87.9
Crude 666 (20-3-77, 666-lignin pitch-pyrophyllite)	5.0	0.55	4	82.2

N.B.—Additional Orvus was not added to any of the above sprays.

Crude 666 was less effective than its gamma isomer content of 10 to 12% would suggest. This may have resulted from the large amount of inert material, both from the original crude 666 and the pyrophyllite or

gypsum used in processing. The slight difference in favour of the 20% crude 666 in gypsum over the same amount in pyrophyllite may have been due to the lignin pitch in the latter mixture causing greater run-off.

Toxicity of 666 to Adult Codling Moths and Oriental Fruit Moths

Adult codling moths were confined in cloth-topped battery jars with apple twigs sprayed with refined 666 (20% ground in pyrophyllite without wetter) at 1 lb. per 100 gal. Females placed with twigs sprayed the same day died within an average of 3.5 days without producing any eggs, while females placed with twigs sprayed two days previously lived an average of 11.7 days and averaged 55.8 eggs. In the check jars, average longevity was 14.3 days and average egg production 57.5 eggs per female.

Lack of moths prevented further tests with codling moth, so adult oriental fruit moths were used to investigate the manner in which 666 acted. When the moths were placed free in battery jars with a sprayed twig, or when they were confined in a wire screen cage within the battery jar so that they could not come in contact with the spray residue, mortality was complete within 24 hours. Longevity of females was also reduced to 4 days, as compared with 8.8 days in the checks, when the moths were confined in battery jars with a gauze-topped can containing dry refined 666. On the other hand, female moths confined with freshly sprayed twigs in open wire screen cylinders in the insectary lived as long as in the checks (an average of 9.9 days in both) and actually deposited a larger number of eggs. Oviposition was very erratic in all cages, however, and the differences were probably not significant.

It is apparent that the vapours given off by 666 spray residues for a short time are very toxic to the adults of both codling moth and oriental fruit moth within a confined space, but it is doubtful whether sprays of 666 would have any appreciable residual effect on the moths under orchard conditions. The effect of actually hitting the moths with 666 sprays was not investigated.

Diamondback Moth (*Plutella maculipennis* (Curt.))

Larvae were raised in mass rearing cultures and those in the last two instars removed for the tests which were conducted in the greenhouse. Two methods of treatment were employed: (a) Larvae on sheets of paper were sprayed for 10 seconds, and at once transferred to untreated pieces of cabbage leaves about 3 sq. in. in area in straight-sided 16-oz. glass jars covered with factory cotton. A strip of moist dental cotton was placed in the jar. This test was for direct contact toxicity. (b) Pieces of sprayed cabbage foliage about 3 sq. in. in area were placed in the jars after the spray had dried, and unsprayed larvae added. This was considered as a stomach poison test, but there was also residual contact action and possibly some fumigant action. In most cases 15 larvae were used per jar and 3 jars per individual test.

The first test indicated that the direct contact action of crude 666 suspensions was much less than that of DDT suspensions, e.g., crude 666 at 4 lb. per 100 gal. gave a kill of 56%, while approximately the same mortality was obtained from 0.25 lb., or one-tenth the amount of DDT. It was shown in another test that 666 was more effective by contact when applied in emulsion form or as a precipitated suspension, than as a suspension made with a wettable spray powder. The emulsion was prepared by diluting 1-200 with water the following stock:

Crude 666—10 grams.
Triton X-100*—10 cc.
Velsicol AR-60†—approx. 86 cc. (to make 100 cc. of solution).

The precipitated suspension was prepared by dilution of a similar stock in which acetone replaced the Velsicol. A proprietary miscible oil solution containing 17% crude 666 was also tested. Dilutions at a rate of 0.5 lb. crude 666 per 100 gal. gave the following results, using 30 larvae per treatment:

Powder suspension	3.3% mortality.
Velsicol emulsion	33.3% mortality.
Proprietary oil emulsion	26.6% mortality.
Acetone suspension	20.0% mortality.

Both DDT and 666 were more effective as stomach poisons than as contact sprays, but again DDT was relatively more toxic than 666. This is shown in Table 2 which is a summary of the data from several tests. Each figure in the table is an average, derived from two or three tests, representing a total of 90 to 135 larvae. It will be noted that the kills by 0.125 lb. gamma 666, 1 lb. crude 666, and 0.062 lb. DDT are approximately equivalent, and similarly the kills for double these amounts of each, hence in these tests DDT was roughly twice as toxic as pure gamma 666, and 16 times as toxic as crude 666.

TABLE 2.—COMPARISON OF CRUDE 666, GAMMA 666, AND DDT AS STOMACH POISONS AGAINST LAST TWO INSTAR DIAMONDBACK MOTH LARVAE

Lb. per 100 gal.	Material and percentage mortality		
	Gamma 666	Crude 666	DDT
	%	%	%
0.031	—	—	50.0
0.062	—	—	53.3
0.125	61.6	12.2	76.6
0.25	71.1	29.4	80.0
0.5	77.7	53.8	93.3
1.0	100.0	53.6	96.6
2.0	100.0	70.5	—
4.0	—	81.0	—

Greenhouse Leaf Tier (*Phlyctaenia rubigalis* (Guen.))

Two tests against the larvae of this species gave almost the same results as obtained with diamondback moth larvae. One test was conducted in the greenhouse with adults, where untreated moths were placed

* A proprietary emulsifier and wetting agent.
† Largely a mixture of methyl naphthalenes.

in (a) an unsprayed wire screen cage with a sprayed plant, and (b) in a sprayed cage with a sprayed plant. Thirty-five adults were used per cage. DDT and gamma 666 were compared at the rate of 1 lb. per 100 gal. The results summarized in Table 3 show that under the conditions of this experiment, gamma 666 was more toxic than DDT, and that as would be expected spraying the cage as well as the plant increased the effectiveness of the treatments.

TABLE 3.—COMPARISON OF DDT AND GAMMA 666 AGAINST ADULT GREENHOUSE LEAF TIERS

Condition of test	Material	Lb. per 100 gal.	No. eggs laid	Length of life; days	
				Max.	Av.
Plant sprayed	DDT	1	388	8	3.8
Plant sprayed	Gamma 666	1	73	2	1.1
Plant and cage sprayed	DDT	1	5	3	1.4
Plant and cage sprayed	Gamma 666	1	40	2	1.0
Check—untreated	—	—	811*	over 8	—

* When observations were discontinued after 8 days about 40% of the moths were still living in the check, so more than 811 eggs would have been laid.

Strawberry Leaf Roller (*Ancylis comptana fragariae* (Walsh & Riley))

This insect, which is much more resistant than diamondback moth and greenhouse leaf tier to DDT, appears to be also quite resistant to 666, the relative difference in toxicity between DDT and 666 appearing about the same as for diamondback moth larvae.

Potted strawberry plants were sprayed in the greenhouse and after the deposit had dried, 14 to 20 nearly mature larvae, collected from the field, were placed on each plant. The data from several tests, summarized in Table 4, indicated that the kill obtained from 2 lb. DDT approximated that from 2 lb. refined 666, but that at the 1-lb. rate DDT was considerably more effective.

TABLE 4.—EFFECT OF DDT AND 666 ON STRAWBERRY LEAF ROLLER LARVAE ON POTTED STRAWBERRY PLANTS

Material	Lb. per 100 gal.	Approx. gamma isomer content; lb. per 100 gal.	No. larvae	No. survived	Mortality
					%
DDT	1	—	160	32	80.0
DDT	2	—	160	18	88.7
DDT	3	—	60	6	90.0
Crude 666	1	0.11	40	31	22.5
Crude 666	2	0.22	40	24	40.0
Refined 666	1	0.35	60	28	53.3
Refined 666	2	0.7	60	11	81.6
American 666	2	0.44	60	23	61.6
American 666	4	0.88	60	9	85.0
American 666	6	1.32	60	9	85.0
Check	—	—	160	138	13.7

Retention of Toxicity by Spray Deposits of DDT and 666

Strawberry plants were sprayed and infested with larvae as soon as the deposit dried. Results were taken 4 days later, and then one week after being sprayed the plants were reinfested. The results were erratic, but, as shown in Table 5, the loss of toxicity by either material was slight, although somewhat greater in the case of 666.

TABLE 5.—COMPARISON OF TOXICITY OF FRESH AND WEEK-OLD DEPOSITS OF DDT AND 666 AGAINST STRAWBERRY LEAF ROLLER LARVAE. SIXTY LARVAE PER TREATMENT

Material	Lb. per 100 gal.	Approx. gamma isomer content; lb. per 100 gal.	Percentage mortality	
			Fresh deposit	1-week-old deposit
			%	%
DDT	1	—	76.6	86.6
DDT	2	—	88.3	83.3
DDT	3	—	90.0	86.6
American 666	2	0.44	61.0	50.0
American 666	4	0.88	85.0	73.4
American 666	6	1.32	85.0	86.6

Asparagus Beetle (*Crioceris asparagi* (L.)) and Spotted Asparagus Beetle (*Crioceris duodecimpunctata* (L.))

In a number of insectary experiments in which untreated adults were placed in cylindrical wire screen cages with sprayed asparagus foliage it was found that DDT was much more effective than 666 against the common asparagus beetle, but apparently somewhat less effective than 666 against the spotted species. For example, as shown in Table 6, 0.125 lb. DDT killed 61% of the former species while the same amount of crude 666 killed 9%, and 0.66 lb. refined 666 killed only 28%. In the case of the spotted

TABLE 6.—EFFECT OF DDT AND 666 ON ASPARAGUS BEETLES. ONE HUNDRED COMMON AND TWENTY-FIVE SPOTTED ASPARAGUS BEETLES PER TEST

Material	Lb. per 100 gal.	Approx. gamma isomer content; lb. per 100 gal.	Percentage mortality	
			Common	Spotted
			%	%
DDT	0.125	—	61.0	—
DDT	0.25	—	93.0	—
DDT	0.5	—	100.0	28.0
DDT	2.0	—	—	64.0
Crude 666	0.125	0.013	9.0	—
Crude 666	0.5	0.055	24.0	—
Crude 666	1.0	0.11	34.0	—
Crude 666	2.0	0.22	45.0	52.0
Crude 666	4.0	0.44	—	52.0
Refined 666	0.33	0.11	14.0	—
Refined 666	0.66	0.23	28.0	52.0
Refined 666	1.32	0.46	—	72.0
Check	—	—	2.0	0.0

species the kill by 0.33 lb. refined 666 was higher than that by 0.5 lb. DDT. Other data in this table show that crude 666 was relatively more effective than refined 666 on the basis of gamma content, e.g., crude 666 at gamma rates of 0.1 and 0.2 lb. per 100 gal. killed 34.0 and 45.0%, respectively of the common asparagus beetle, while refined 666 at the same gamma rates killed 14.0 and 28.0%, respectively.

Striped Cucumber Beetle (*Diabrotica vittata* (F.))

Adults of this species were shown to be very susceptible to both DDT and 666 when placed in cages with sprayed plants. DDT at the lowest rate tested, 0.125 lb. per 100 gal., killed 100%, while crude 666 at 0.125, 0.25 and 0.5 lb. per 100 gal. killed 71.5, 88.5, and 94.5%, respectively. Refined 666 at 0.5 lb. per 100 gal. gave 100% mortality.

An estimate of the amount of foliage consumed by the beetles showed better protection by DDT than by 666, e.g., the proportion consumed of foliage sprayed with crude 666 at 0.125, 0.25 and 0.5 lb. per 100 gal. being 5, 20 and 30%, respectively; and for DDT at the same rates it was trace, 1 and 3%, respectively.

Rose Chafer (*Macrodactylus subspinosus* (F.))

A number of tests were conducted in the insectary in July but the results were unsatisfactory owing to the age of the beetles which resulted in mortalities of 50 to 60% in the checks during the observation period. However, it is worth noting that crude 666 appeared to be about equally as toxic as DDT, e.g., in a test at rates varying from 0.062 to 0.25 lb. per 100 gal. both crude 666 and DDT killed approximately 85% at the lowest rate and 100% at the highest, while the mortality from lead arsenate at 5 lb. per 100 gal. was only 75%.

Pear Psylla (*Psylla pyricola* Foerst.)

To determine the action of 666 on pear psylla nymphs, infested potted plants or infested shoots from the orchard which bore nymphs of all instars were used. From the time of spraying to examination, a period of from 3 to 5 days, sprayed and check plants were kept unconfined in the insectary.

Adults had to be caged, and since they are able to pass through 16-mesh screening, either 40-oz. wide-mouth glass containers or 32-mesh wire gauze cages were used. In all instances sprayed twigs were allowed to dry before being put in the cages with adults.

Relative Toxicity of DDT and 666 Against Nymphs

An initial test, the results of which are summarized in Table 7, showed that crude 666 was much more effective than DDT, a powder suspension spray of the latter at 1 lb. per 100 gal. killing only 16.7% as compared with 91.2% by 0.25 lb. of crude 666. It is interesting to note that a powder suspension of DDT is less effective as a contact spray than an emulsion (Velsicol) or precipitated suspension spray (acetone) of DDT.

TABLE 7.—COMPARISON OF DDT AND 666 SPRAYS AGAINST PEAR PSYLLA NYMPHS

Materials	Lb. per 100 gal.	No. of tests	Total nymphs	Percentage mortality
DDT (powder suspension)	1	3	1467	16.7
DDT (Velsicol emulsion)*	1	3	1342	51.9
DDT (precip. acetone suspension)†	1	3	1234	43.0
Crude 666	1	4	1974	99.9
Crude 666	0.5	6	1998	88.6‡
Crude 666	0.25	2	728	91.2
Checks	—	5	1483	4.5

* and † Prepared by diluting: DDT 20 gm.
Triton X-100 10 cc.
Velsicol AR-60 or
Acetone to make 100 cc.

‡ Living nymphs were largely those newly hatched from eggs present before spraying.

In a series of dosage tests, summarized in Table 8, it was found that crude 666 at a rate as low as 0.25 lb. per 100 gal. gave a kill of over 85%, and refined 666 at 0.2 lb. per 100 gal. gave a similarly high mortality. It appears that crude 666 is relatively more toxic than its gamma isomer content would indicate.

TABLE 8.—DOSAGE TESTS OF 666 ON PEAR PSYLLA NYMPHS

Materials	Lb. per 100 gal.	Approx. gamma isomer content; lb. per 100 gal.	No. tests	Total nymphs	Percentage mortality
Crude 666	2	0.22	2	929	100.0
Crude 666	1	0.11	5	3012	99.5
Crude 666	0.5	0.05	4	1589	96.8
Crude 666	0.25	0.027	3	1577	86.9
Crude 666	0.2	0.022	1	644	51.5
Refined 666	1	0.35	2	681	97.1
Refined 666	0.5	0.175	8	5726	98.2
Refined 666	0.25	0.087	2	514	79.6
Refined 666	0.2	0.07	2	735	86.9
Refined 666	0.1	0.035	2	612	55.1
Checks	—	—	10	5751	4.3

Residual Action of 666 on Pear Psylla Nymphs

Small potted seedlings bearing psylla eggs were sprayed with various strengths of 666 at periods when the eggs were at different stages of development. No cages were used on the plants after the sprays were applied. When the psyllas had reached the first and second instars, examinations were made to determine if the sprays applied before hatching had destroyed the nymphs.

The results given in Table 9 showed that 666 sprays had no apparent lethal action on the eggs but killed large percentages of nymphs hatching from eggs present on sprayed foliage. Sprays applied nearest to the time of hatching were more effective than those applied earlier.

TABLE 9.—RESIDUAL ACTION OF 666 ON PEAR PSYLLA NYMPHS

Materials	Lb. per 100 gal.	Approx. gamma isomer content; lb. per 100 gal.	Age of eggs* when sprayed	Total nymphs	Percentage mortality
			days		
Crude 666	1.0	0.11	1– 7	310	85.5
			3–10	291	92.8
			7–10	223	96.8
Crude 666	0.5	0.055	1– 7	368	58.7
			3–10	220	83.3
			7–10	251	88.9
Crude 666	0.25	0.027	1– 7	296	50.6
			2–10	320	70.6
			7–10	312	42.2
Refined 666	1.0	0.35	7–10	134	95.5

* Total incubation period was 10 to 12 days.

Fumigant Action of 666

Table 10 shows that 666 has a pronounced fumigant action on adult pear psyllas within a confined space. Adults enclosed in cloth-covered glass containers with crude 666, but separated from it by wire gauze, were all killed within a 6-day period, and laid an average of 1.3 eggs per psylla. When a fine mesh wire gauze cage was used instead of the glass jar to allow air circulation, the mortality was greatly reduced and large numbers of eggs were deposited. However, adults confined on sprayed twigs succumbed as rapidly in a gauze cage as in a glass container.

TABLE 10.—FUMIGANT ACTION OF CRUDE 666 ON PEAR PSYLLA ADULTS

Method of use	Total adults	Percentage mortality after:			Total eggs laid	Average no. eggs per adult
		1 day	3 days	6 days		
		%	%	%		
Dry crude 666 (10 gm.) placed in bottom of quart glass jar and separated by fine wire gauze from psyllas and pear twig placed above	23	74.0	82.6	100.0	29	1.3
As above, but wire gauze cage used in place of glass jar	23	0.0	17.4*	17.4*	1009	44.0
Check; as above, with wire gauze cage but without 666	21	0.0	0.0	0.0	1440	68.9
Twig sprayed with 1 lb. crude 666 per 100 gal. in glass jar with psyllas	44	100.0	—	—	0	0
Check; as above but twig not sprayed	40	7.5	12.5	35.0	496	12.4
Twig sprayed with 1 lb. crude 666 per 100 gal. in wire gauze cage with psyllas	25	100.0	—	—	7	0.3
Check; as above but twig not sprayed	20	0.0	0.0	0.0	216	10.8

* Partly estimated because of poor visibility through fine gauze.

Persistence of Toxicity of 666 Residues

Pear twigs in jars of water were sprayed with crude 666 at 1 lb. per 100 gal., and at daily intervals thereafter placed in cloth-covered glass containers along with pear psylla adults.

The results in Table 11, especially those taken one day after caging, show a general trend of decreasing mortality and higher egg deposition as the interval after spraying was increased. There might have been fewer inconsistencies in the final records if lower rates of 666 had been used. It should be noted that the psyllas were confined in glass containers so that the 666 could act both as a fumigant and as a contact insecticide.

TABLE 11.—PERSISTENCE OF RESIDUAL ACTION OF CRUDE 666 1 LB. PER 100 GAL. ON PEAR FOLIAGE AGAINST ADULT PEAR PSYLLA

Age of spray deposit; days	No. of tests	Total adults	Percentage mortality after:				Final records after 7 days:	
			1 day	3 days	5 days	7 days	Total eggs laid	Aver. no. eggs per adult.
			%	%	%	%		
Fresh	2	50	100.0	100.0	100.0	100.0	0	0.0
1	2	56	69.6	100.0	100.0	100.0	99	1.8
2	5	120	25.8	61.7	87.4	95.8	1221	10.2
3	3	76	35.5	81.7	90.8	100.0	302	4.0
4	1	21	38.1	95.2	100.0	100.0	142	6.8
5	4	90	5.6	46.7	85.5	100.0	878	9.8
6	1	24	4.2	54.2	79.0	91.7	671	28.0
Checks	9	206	2.4	6.8	23.3	38.3	5058	24.6

Compatibility of 666 with Fungicides in Pear Psylla Sprays

Spray combinations of 666 with some common fungicides were as effective against pear psylla nymphs as 666 alone (Table 12). Refined 666 was used at 0.5 lb. per 100 gal., and it should be pointed out that as all the resulting kills were close to 100%, the possible deleterious effect of some of the fungicides may have been masked, although it is clear that from a practical standpoint this is unlikely to be of much significance.

TABLE 12.—EFFECT OF FUNGICIDES ON THE ACTION OF 666 AGAINST PEAR PSYLLA NYMPHS

Materials used per 100 gal.	Total nymphs	Percentage mortality
Refined 666, 0.5 lb. with:		
Bordeaux mixture 5-10-100	815	99.3
Dry flotation sulphur 10 lb.	562	97.2
Fixed copper (COCS)* 3 lb. with hydr. lime 3 lb.	709	97.3
Fermate†, 2½ lb.	614	99.8
Refined 666, 0.5 lb. used alone	650	98.6
Check—Not sprayed	745	4.2

*Copper oxychloride sulphate.
† Ferric dimethyldithiocarbamate.

Green Chrysanthemum Aphid (*Rhopalosiphum rufomaculatum* (Wilson))

DDT and 666 were tested against this insect by two methods: (a) plants infested with aphids were sprayed and mortality counts made 4 days later; and (b) uninfested plants were sprayed and after the deposit dried, 10 adults added per plant and the population recorded 9 days later. As a direct contact spray, crude 666 was about twice, and gamma 666 about 8 times as toxic as DDT, when all three were used as powder suspensions. It is interesting to note in Table 13 that the pure gamma 666 was only approximately 4 times as toxic as the crude 666, although the latter contained only 10 to 12% of the gamma isomer.

TABLE 13.—EFFECT OF DDT AND 666 ON GREEN CHRYSANTHEMUM APHID AS DIRECT CONTACT SPRAY ON INSECTS AND PLANTS

Lb. per 100 gal	Gamma 666		Crude 666		DDT	
	No.	Mort.	No.	Mort.	No.	Mort.
		%		%		%
0.031	587	52.5	—	—	1065	4.0
0.062	939	84.6	1765	10.0	743	10.2
0.125	1005	97.7	2197	38.6	514	38.9
0.25	882	99.6	941	61.1	149	75.2
0.5	—	—	964	94.7	—	—
1.0	—	—	547	99.6	—	—

(Check 7.8% mortality)

In the test for residual effect, where the plants only were sprayed, DDT was considerably more effective than 666. From an initial population of 30 adults per treatment the following populations resulted after 9 days: check, 216 aphids; crude 666 at 0.25, 0.5 and 1.0 lb. per 100 gal., 123, 40, and 4 aphids, respectively; and DDT at 0.25, 0.5 and 1.0 lb. per 100 gal., 9, 4, and 0 aphids, respectively. This may indicate more rapid loss of toxicity by 666 on the foliage.

Squash Bug (*Anasa tristis* (DeG.))

DDT and 666 were tested against this insect by placing adults in small cylindrical screen cages, and dusting them fairly heavily, using a small hand duster and giving the same number of puffs for each treatment. A circular slice of gourd or squash about 3 in. in diameter was similarly dusted, then the insects and the piece of gourd or squash transferred to clean cages over moist sand in flower pot saucers. The standard formula wettable spray powders (10% DDT or 666, 3% Orvus, 87% pyrophyllite) were used as dusts in most cases, diluted with pyrophyllite when necessary. An American sample of 5% 666 dust was also used.

The observation cages used for the first test were 4 in. in diameter and 5 in. high, and the kill obtained was considerably higher than in those subsequently used which were 8 in. in diameter and 1 foot high. This

difference was probably due to the fact that in the smaller cages the bugs spent more time on the treated gourd or squash, and in the case of the 666 this almost undoubtedly resulted in increased fumigant action.

In both the large and small cages refined 666 gave a more rapid and higher kill than an equal amount of DDT. Five days after treatment the results in the small cages were as follows: 10, 5 and 2.5% refined 666, 100% mortality in all cases; DDT dusts of the same concentration, 100, 83.3 and 91.6% mortality, respectively; and for the check 0%. The more rapid kill by 666 is indicated by the mortality figures two days after treatment, which were 100, 100 and 91.6%, respectively for the three 666 dusts, and 50.0, 25.0 and 8.3%, respectively for the DDT dusts.

The experiment in the large cages was a comparison of two samples of 666 with DDT. The two 666 materials, refined 666 from England, and the American sample of 666 (22% gamma isomer) were diluted to give approximately the same gamma content. The results given in Table 14 show somewhat higher kill by refined 666 from England than by the American sample, but this may be because the dilution of the refined 666 was based on a gamma content of 30%, whereas it may actually have been nearer 40%, the manufacturer having stated the gamma content to be between 30 and 40%.

TABLE 14.—COMPARISON OF DUSTS OF 666 AND DDT AGAINST ADULT SQUASH BUGS. TWENTY-FIVE BUGS PER TREATMENT

Material	Approx. gamma isomer content	Percentage mortality
	%	%
3.6 % refined 666 dust	0.1*	100
1.8 % refined 666 dust	0.55*	76
0.9 % refined 666 dust	2.275*	68
0.45% refined 666 dust	0.137*	44
5 % 666 dust (American sample)	0.1	76
2.5 % 666 dust (American sample)	0.55	68
1.25% 666 dust (American sample)	0.275	60
0.62% 666 dust (American sample)	0.137	24
10 % DDT dust	—	76
5 % DDT dust	—	67
2.5 % DDT dust	—	68
1.25% DDT dust	—	20

* Calculated on basis of 30% gamma content for refined 666.

Large Milkweed Bug (*Oncopeltus fasciatus* (Dall.))

This species was used as a test insect because it can easily be reared in large numbers on dry milkweed seed during the winter. Cylindrical test cages 4 in. in diameter and 5 in. high were made of 16-mesh wire screen and placed on racks so that air could enter through the bottom as well as through the top and sides. Each cage was provided with a tablespoonful of milkweed seeds and a moist cotton wick. As the bugs are very active,

they were immobilized by being chilled for 15 minutes in a vial plunged in ice-water, after which they were sprayed for exactly 10 seconds and at once transferred to the observation cages, 50 bugs per cage. The first test indicated that refined 666 was more than twice as toxic as DDT under these conditions, the kills for refined 666 at 0.025, 0.5, 1.0 and 2.0 lb. per 100 gal. being 70, 90, 100 and 100%, respectively after 6 days, while kills by the same rates of DDT were 3.0, 11.0, 30.0 and 72.0%, respectively.

Fumigant Action of 666

Because it was suspected that 666 was acting as a fumigant in these cages an experiment was set up in which the insects were exposed to a variety of conditions as described in Table 17, and the following points were brought out: (a) The fumes from the refined 666 spray deposit on 50 dead bugs were sufficiently toxic to kill 50 unsprayed bugs in a sealed jar, but had little or no effect in wire screen cages. (b) The same deposit on the living bugs gave a kill approaching 100% in the wire screen cage in still air, but only 5% mortality after 4 days when the cage was placed in a breeze of approximately 8 m.p.h. from a fan. (c) A spray deposit which was rapidly dried with a fan in about 5 minutes appeared to be just as toxic later in still air as one allowed to dry slowly, but a deposit kept in the breeze for 4 days appeared to have lost its toxicity entirely.

TABLE 17.—TOXICITY OF REFINED 666 1 LB. PER 100 GAL. TO MILKWEED BUGS UNDER VARIOUS CONDITIONS IN CAGES. FIFTY BUGS PER CAGE

Conditions of experiment	Percentage mortality 4 days after treatment
1. Wire screen cage in breeze from fan	5
2. Wire screen cage in still air	91
3. Wire screen cage, bugs dried quickly, then placed in still air	88
4. Fifty unsprayed bugs placed in wire screen cage containing 50 sprayed dead bugs	6
5. Fifty unsprayed bugs placed in sealed pint glass jar with 50 dead sprayed bugs	100
6. Same as 5, but dead bugs dried before being placed in glass jar	100
7. Checks, sealed jar and wire cages	0

N.B.—The dead bugs in 4, 5, and 6, were separated from the living bugs by a small wire cage within the observation cage.

Three series of tests were conducted in which the cages were placed at various distances from the fan and the wind velocity determined. Anemometer reading showed that speed of the fan varied somewhat with changes in the electric current. The insects were chilled, then sprayed for 7 seconds and at once transferred to the cages, 50 adults per cage. The spray gun delivery was greater than for the previously described tests, and this may have accounted for the relatively higher kills.

The results from the three series of tests are presented in Table 18 and show a marked reduction in the speed of kill as the wind velocity increased, although at the end of one week the differences between cages were not as great as obtained in the earlier tests. These experiments suggest that careful observations should be made in field experiments to note differences due to temperature, humidity, and particularly wind.

TABLE 18.—EFFECT OF WIND VELOCITY ON CONTACT ACTION OF REFINED 666 AT 1 LB. PER 100 GAL. AGAINST ADULT MILKWEED BUGS. FIFTY BUGS PER CAGE

Wind velocity m.p.h.		Days after treatment—percentage mortality						
Outside cage	Inside cage	1	2	3	4	5	6	7
		%	%	%	%	%	%	%
Test A								
6.91	6.34	4	8	20	—	—	—	—
6.11	5.09	4	14	48	—	—	—	—
2.36	2.04	20	30	62	—	—	—	—
1.44	0.45	62	80	90	—	—	—	—
1.06	Trace	62	80	86	—	—	—	—
0.79	Trace	36	68	76	—	—	—	—
Trace	Trace	78	84	94	—	—	—	—
Zero	Zero	100	100	100	—	—	—	—
Test B								
3.05	1.74	24	24	26	60	80	94	96
1.90	1.25	28	28	34	70	90	96	98
1.27	0.92	50	52	76	86	90	94	94
0.81	Trace	72	78	84	98	98	100	100
Trace	Trace	72	88	96	98	98	100	100
Trace	Trace	78	92	92	96	98	100	100
Trace	Trace	96	100	100	100	100	100	100
Trace	Trace	100	100	100	100	100	100	100
Test C								
8.31	5.86	8	24	52	70	80	86	88
4.72	2.76	10	36	72	76	78	80	86
2.24	0.85	18	54	82	88	96	96	98
1.05	0.11	82	90	98	100	100	100	100
0.45	Trace	84	90	96	96	98	98	98
Trace	Trace	86	96	96	96	98	98	98
Zero	Zero	88	100	100	100	100	100	100

Unsprayed check in still air—zero mortality after one week

Chrysanthemum Thrips (*Thrips nigropilosus* Uzel.)

A few observations made on the control of these thrips on the chrysanthemum plants sprayed for aphid tests, indicated that crude 666 is toxic to this species, but less so than DDT, e.g., all nymphs were eliminated by DDT at rates as low as 0.125 lb. per 100 gal. (wettable spray powder suspension), while a similar spray of crude 666 at the same rate reduced the population by approximately 80%. No information was obtained on the residual effect of 666.

European Red Mite (*Paratetranychus pilosus* (C. & F.))

No test with 666 was made specifically against this mite, but thriving populations developed in the insectary on potted apple seedlings which had been sprayed with 666 at high concentrations.

Common Red Spider Mite (*Tetranychus telarius* (Linn.))

In a test where crude 666 was used at 0.25, 1.0, and 4 lb. per 100 gal. kills of red spider mite of 3.2, 21.4, and 43.9%, respectively resulted on rose plants, while pure gamma 666 at 0.5 and 1 lb. per 100 gal. killed 50.1 and 72.1%, respectively on cucumber, indicating that the material is of little value against this mite.

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INFLUENCE OF DDT ON YEAST FERMENTATION[1]

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The Dominion Entomological Laboratory at Vineland, Ontario, carries on extensive experiments on the control of insect pests with the aid of dichloro-diphenyl-trichlorethane, commonly known as DDT. One of their problems is the control of insect pests in the vineyard. Naturally they were interested in knowing if DDT has a detrimental effect on yeast fermentation. Therefore at the request of W. A. Ross, Chief, Fruit Insect Investigations, this experiment was carried out.

In a comparatively short time a voluminous literature has been accumulated on the subject of control of insect pests by use of DDT sprays. However, so far as the author is aware, there is no published record dealing with the influence of DDT on yeast fermentation. Surveying the literature on the subject of the activity of DDT against microorganisms it was found that Granovsky (1), working on control of insects on potato plants, reported that plants dusted with DDT were somewhat less affected by leaf parasitic fungi than the plants of control plots. Morris (2), however, carrying experiments *in vitro* and with soil under greenhouse conditions, was unable to control the growth of *Ophiobolus graminis*, *Colletotrichum trifolii*, *Ascochyta imperfecta*, *Pleospora herbarium*, *Pseudoplia trifolii*, *Urocystis tritici* and damping off organisms with the aid of DDT. Furthermore, Abbott (3) reported that 105 mg./ml. of DDT in the medium did not affect the growth of *Physarella oblonga*. However, when DDT was applied in powdered form on the surface of the medium the growth of the same organism was to a certain extent delayed as compared with the growth on control plates. The delay in growth was attributed to mechanical reasons.

In this laboratory previous experiments indicated that 217 mg. per cent DDT in the medium did not affect the growth of *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Blastomyces Gilchristi*, *Microsporon lanosum* and *Trichophyton gypseum*. Furthermore, it was noticed that when required amounts of DDT were dissolved in ethanol and added to an agar medium, crystals formed in the majority of cases, after evaporation of alcohol and cooling of agar. In these cases the fungi and bacteria were able to grow normally in direct contact with crystals of DDT. Negative results were also obtained when DDT was added to the medium in oil solutions (mineral and vegetable, soybean). In this case the organisms were able to grow (mycelium) through or on the oil droplets.

In this report the influence of DDT on yeast fermentation is presented and discussed.

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Experiment 1

RESULTS

The organism *Saccharomyces ellipsoideus* was grown in the following medium: urea, 0.1 gm.; grape juice concentrate, 30.0 gm.; commercial refined cane sugar, 19.3 gm.; distilled water, 171 ml. Fifteen flasks, each containing 200 ml. of the medium, were prepared, 9 flasks receiving the following amounts of DDT:

Flask No.	Weight of powder	Weight of DDT
	gm.	gm.
1	0.0210	0.0021
2	0.0420	0.0042
3	0.0630	0.0063
4	0.0940	0.0094
5	0.1050	0.0105
6	0.1580	0.0158
7	0.2100	0.0210
8	0.3150	0.0315
9	0.4200	0.0420

The powder consisted of diatomaceous earth containing 10% DDT ground in a ball mill to colloidal texture.

Six flasks were kept as controls. After addition of DDT the flasks were autoclaved at 15 lb. pressure for 20 minutes, cooled and inoculated with 5 ml. yeast suspension, except the last 2 flasks (14 and 15) which were kept as uninoculated controls. The flasks were then weighed and incubated at 22 to 24° C.

The original sugar content in the media was determined by refractometer, and as fermentation progressed the sugar content was maintained at 7% by the constant weight method by addition of 50% sugar syrup. The incubation was discontinued on the 10th day and results of this experiment are summarized in Tables 1, 2, and 3. The results obtained do not indicate outstanding differences as among DDT treated media if compared with control media. If, however, we compare the average daily loss in weight per day of 9 DDT treated media with average daily loss of control media (Table 2) we note that the intensity of fermentation during the first 5 days of incubation was depressed by the addition of DDT (weighing began on the 3rd day of incubation). However, during the last 5 days of incubation the fermentation was more vigorous in DDT treated media than in controls.

Data presented in Table 1 also indicate the considerable variation in yield of alcohol as expressed in per cent by volume. However, the general trend in yield of alcohol indicates a gradual decrease in yield with increasing amounts of DDT added to the medium. The average yield of alcohol per flask of DDT treated media is lower than the average yield in control media. However, if DDT treated media are divided into two lots, the first consisting of first 4 flasks to which the amount of DDT added was from 2 to 9 mg. and second lot from 10 to 42 mg. per 200 ml. of medium, and the average yield of alcohol produced compared, we then note that the production of alcohol in the first lot is equal to that of the control media,

TABLE 1.—EFFECT OF DDT ON DAILY AND TOTAL CONSUMPTION OF SUGAR DURING FERMENTATION AND ON ALCOHOL PRODUCTION

Treatment	Time of incubation—days: Loss in weight—grams								Total loss per sample, gm.	Average loss, gm.	Total sugar consumed, gm.	Average sugar consumed, gm.	Yield alcohol percentage by volume	Average yield of alcohol percentage by volume
	3	4	5	6	7	8	9	10						
DDT added														
1	0.50	0.80	4.55	2.75	4.70	1.00	0.20	0.15	14.65		34.15		10.86	Average of
2	0.55	1.10	5.45	2.55	4.35	0.80	0.20	0.15	15.15		34.51		10.80	4 treatments
3	0.65	1.50	5.50	2.85	3.70	0.40	0.15	0.15	14.90		34.33		10.86	10.80 10.68
4	0.80	1.75	6.20	2.85	2.95	0.25	0.15	0.10	15.05		34.34		10.69	
5	0.95	2.00	6.65	2.70	2.65	0.20	0.00	0.05	15.20	15.05	34.49	34.41	10.61	Average of
6	0.85	1.65	5.90	3.55	2.65	0.25	0.05	0.00	14.90		34.40		10.63	5 treatments
7	0.50	1.50	6.40	2.95	3.60	0.20	0.15	0.00	15.30		34.59		10.61	10.58
8	0.35	1.05	5.90	2.75	4.55	0.35	0.15	0.05	15.15		34.44		10.61	
9	0.45	1.35	6.20	2.60	4.10	0.30	0.10	0.05	15.15		34.44		10.44	
Control inoculated														
10	0.80	1.40	5.20	2.35	3.95	0.80	0.15	0.10	14.75		34.18		10.69	
11	1.40	2.15	5.75	2.45	3.05	0.30	0.20	0.20	15.50		34.79		10.81	
12	0.70	1.70	7.30	2.45	3.00	0.15	0.15	0.05	15.50	15.21	34.86	34.55	10.86	10.80
13	0.75	1.65	6.75	2.60	2.85	0.30	0.20	0.00	15.10		34.39		10.84	
Control not inoculated														
14	—	—	—	—	—	—	—	—	—	—	—	—	—	
15	—	—	—	—	—	—	—	—	—	—	—	—	—	

Original sugar content of medium = 17.1% by volume.
Sugar content in fermented juice = 7% by volume.

TABLE 2.—EFFECT OF DDT ON DAILY LOSS IN WEIGHT OF MEDIA DURING FERMENTATION

Incubation days	DDT treated media		Control media	
	Average loss—gm.		Average loss—gm.	
	Daily	5 Days	Daily	5 Days
3	0.62		0.91	
4	1.41		1.72	
5	5.86	1.58	6.25	1.76
6	2.84		2.46	
7	3.69		3.21	
8	4.17		3.90	
9	0.22		0.18	
10	0.08	2.20	0.09	1.89

TABLE 3.—EFFECT OF DDT ON ALCOHOL PRODUCTION COEFFICIENT*

Treatment	Sugar consumed, gm.	Volume fermented juice, ml.	Yield of alcohol, ml.	Alcohol production coefficient	
				Per treatment	Average
1	34.15	210	22.81	66.79	4 treatments
2	34.51	212	22.90	66.36	66.55
3	34.33	211	22.92	66.73	
4	34.34	213	22.77	66.31	
5	34.49	213	22.60	65.53	5 treatments
6	34.40	210	22.32	64.88	65.17
7	34.59	213	22.60	65.34	
8	34.44	213	22.60	65.62	9 treatments
9	34.44	213	22.24	64.58	65.79
10	34.18	211	22.56	66.00	
11	34.79	213	23.03	66.20	66.35
12	34.86	212	23.02	66.04	
13	34.39	213	23.09	67.14	

* $K = \frac{\text{total yield of alcohol, ml.}}{\text{total consumption of sugar—grams}} \times 100.$

whereas production of alcohol in the second lot is somewhat lower than in the controls. Furthermore, if the "production coefficient" is calculated from data obtained, that is production of total alcohol ml., per gram of sugar consumed, it will be seen (Table 3) that the production coefficient is gradually decreased with increasing amounts of DDT added. However, if the average production coefficient is calculated for the first 4, and for the last 5 DDT treated media, and compared with the average production coefficient of the controls, it will be evident that production coefficient of the first lot is higher and that of the second lot lower than the average production coefficient of control flasks. This would indicate that addition of not more than 10 mg. of DDT per 200 ml. of medium will create a condition more economical for production of alcohol.

Experiment 2

Grapes sprayed with DDT and grapes sprayed according to the regular schedule with lead arsenate, were received from the Entomological Laboratory, Vineland, Ontario, for experimental purposes. The plots had been sprayed three times during the season, the rate of application being $1\frac{1}{2}$ half lb. of actual DDT per acre of vineyard. Therefore the total weight of actual DDT in three sprays amounted to $4\frac{1}{2}$ lb. per acre.

It was impossible to arrange the experiments immediately on arrival of the shipment. For this reason the grapes were stored in the basement for 15 days prior to using them in experiments.

On examination of the samples after storage and during the preparation for fermentation it was noticed that DDT sprayed grapes were less damaged or contaminated with moulds (chiefly *Penicillia*, *Phycomycetes* and *Aspergilli*) than the control sample.

Samples were prepared for fermentation as follows: (1) 2600 gm. grapes from each treatment, in duplicate, were crushed by hand and deposited separately into 10-l. flasks; (2) each flask received 370 gm. refined cane sugar and 1477 ml. of tap water. Each flask was inoculated with 100 ml. actively fermenting medium. The inoculum was started in sterile medium of the same composition as in the case of the first experiment, by inoculation with *Saccharomyces ellipsoideus*. Sugar content of the fermenting grape juice was determined by the Brix method, by subtracting 2 from the Brix reading for non-sugar soluble solids. The sugar content of the fermenting juice was maintained at 7% throughout the experiment by addition twice daily (early stage of fermentation) of cane sugar. The flasks were incubated at room temperature, with daily fluctuation of temperature between 70 and 80° F. Weights of fermenting juice and weights of daily addition of sugar are presented in Tables 4 and 5.

At the end of fermentation (13 days) total volume and weight of fermented juice of each sample were determined. Alcohol per cent by volume, and per cent of total and volatile acids were determined by the official A.O.A.C. methods. Results are summarized in Tables 4, 5 and 6.

The average percentage of alcohol by volume and total yield of alcohol per cent by volume produced by DDT sprayed grapes are lower than in the control samples, whereas the percentages of total and volatile acids are slightly higher, 0.015 and 0.003, respectively, than in control samples.

The intensity of fermentation, as indicated by daily addition of sugar, in flasks with DDT sprayed grapes was to a certain degree retarded at the beginning of the experiment, and was more vigorous at the end of the experiment as compared with the control grapes. The alcohol production coefficient for DDT sprayed grapes is again higher than for control grapes. In general the results of this experiment are in agreement with results obtained from the first experiment, except that the production coefficient in this case is only approximately half as high as in the first experiment. The content of DDT in fermented grape juice has been determined by the Division of Chemistry, Department of Agriculture; according to their report no DDT was found in the fermented juices. The sensitivity of the method used was less than 2 mg. per 100 ml. of the fermented grape juice.

TABLE 4.—DAILY LOSS IN WEIGHT OF MEDIA WITH DDT SPRAYED GRAPES AND MEDIA WITH GRAPES SPRAYED WITH LEAD ARSENATE (CONTROL)

Treatment	Time of incubation—days													Total loss
	1	2	3	4	5	6	7	8	9	10	11	12	13	
	Daily loss—grams													gm.
DDT spray I	0	46	203	69	115	184	138	10	112	50	65	25	24	1041.0
DDT spray II	0	124	226	55	148	184	138	56	89	10	106	35	20	1191.0
Average	0	85	214.5	62	131.5	184	138	33	100.5	30	85.5	30	22	1116.0
Control I	156.3	134	194	78	102	115	115	33	20	80	56	10	13	1106.3
Control II	78.2	202	194	78	125	125	125	56	66	50	60	20	10	1189.2
Average	117.3	168	194	78	113.5	120	120	44.5	43	65	58	15	11.5	1147.8

TABLE 5.—CONSUMPTION OF SUGAR IN FERMENTATION OF DDT SPRAYED GRAPES AND GRAPES SPRAYED WITH LEAD ARSENATE (CONTROL)

Treatment	Before fermentation			After fermentation			Sugar added during ferment., gm.	Total sugar consumed, gm.
	Wt. of sample, gm.	Sugar percentage (Brix)	Calcul. wt. of sugar per sample, gm.	Wt. of ferment. juice, gm.	Sugar percentage (Brix)	Sugar not ferment., gm.		
DDT spray I	4600	18.0	828.0	4110	6.30	258.9	1041.0	1610.1
DDT spray II	4600	17.8	818.8	4250	6.20	263.5	1191.0	1746.3
Control I	4600	17.5	805.0	4210	6.20	261.0	1106.3	1650.3
Control II	4600	17.6	809.6	4250	6.55	278.4	1189.2	1720.4

TABLE 6.—ALCOHOL PRODUCTION BY FERMENTATION OF DDT SPRAYED GRAPES AND GRAPES SPRAYED WITH LEAD ARSENATE (CONTROL)

Treatment	Volume fermented, juice, ml.	Total acids, %	Volatile acids, %	Alcohol percentage by volume	Yield alcohol, ml.		Sugar consumed, gm.	Alcohol product. coefficient*	
					Per treat.	Average		Per treat.	Average
DDT spray I	4180	0.61	0.070	13.34	557.6	555.25	1610.1	34.63	33.15
DDT spray II	4280	0.60	0.070	13.01	552.9		1746.3	31.66	
Control I	4210	0.58	0.067	13.35	562.1	556.85	1650.3	34.06	33.06
Control II	4220	0.61	0.067	13.07	551.6		1720.4	32.06	

* $K = \frac{\text{Yield of alcohol, ml.}}{\text{Sugar consumed—grams}} \times 100.$

DISCUSSION

The results of the first experiment indicate no outstanding difference in the production of alcohol or consumption of sugar during the process of fermentation due to the addition of different amounts of DDT to the medium. Furthermore, no striking differences have been obtained in the production of alcohol by DDT treated and control media. However, minor differences, such as lag in intensity of fermentation during the first 5 days of incubation in DDT treated medium, slightly lower production of alcohol in per cent by volume or slight differences in production of alcohol per gram of sugar consumed, were evident.

Because of the fact that a control with diatomaceous earth alone was not included in the first experiment, there is no definite proof that the delay in the intensity of fermentation was due to the mechanical or chemical effect of DDT or due to the mechanical action of diatomaceous earth. However, when grapes sprayed with DDT were used for alcohol production, a similar trend of fermentation was noted. In this case diatomaceous earth was not included in the spray formulae. Therefore the delay in intensity of fermentation may be attributed to the influence of DDT in powder or in spray form on the intensity of fermentation during the first 5 days incubation.

SUMMARY

An investigation has been made of the effect of DDT on yeast fermentation by comparing the volume of alcohol produced by the yeast in DDT treated and control media. The production of alcohol from DDT sprayed grapes in comparison with alcohol production from control (sprayed with lead arsenate) grapes was also investigated.

DDT treated media containing from 5 to 20 mg. per cent of actual DDT showed 2.96% reduction of alcohol production, expressed in per cent by volume, and 1.96% reduction in total yield of alcohol, if the production of alcohol in control media was taken as 100. However, when media contained less than 5 mg. per cent DDT the production of alcohol was the same as in control media.

Production of alcohol per gram of sugar consumed was not affected by the addition to the medium of up to 5 mg. per cent of actual DDT, whereas addition of more than 5 and up to 21 mg. per cent, resulted in 1.78% reduction when production in control media is expressed as 100.

The intensity of fermentation in DDT treated media was retarded during the first 5 days incubation; however, this delay was largely compensated for during the following days of incubation.

In general similar results have been obtained when grapes sprayed with DDT for control of insect pests were used for alcohol production.

According to chemical analyses no DDT was detected in the fermented grape juice obtained from DDT sprayed grapes.

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ALFALFA SEED PRODUCTION IN NORTHERN SASKATCHEWAN AS AFFECTED BY LYGUS BUGS, WITH A REPORT ON THEIR CONTROL BY BURNING[1]

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In the period 1941-44 a series of investigations was carried out on the effects of various insects on alfalfa seed production in the White Fox district of northern Saskatchewan. Other publications (4, 5) have discussed relationships between honey bees and wild bees, and seed setting; these contained a general description of the history and natural vegetation of the area. This study was intended to deal with injurious insects belonging to the genera *Adelphocoris* Reut. and *Lygus* Hahn[4]. Members of both genera have been reported as attacking alfalfa in the northern portions of nearctic America. Hughes (2) found *A. lineolatus* Goeze and to a much lesser extent *A. rapidus* Say, of prime importance in northern Minnesota, and their prevalence in the White Fox area should be mentioned. However, *A. lineolatus* was not found and *rapidus*, although widely distributed throughout the area, was not numerous enough to be considered of economic importance. Hence, further reference will concern *Lygus* only, and particularly *L. hesperus* Knt. and *L. elisus* Van D.

DESCRIPTION AND DISTRIBUTION

An excellent description of these two species of *Lygus* is given by Sorenson (9). The adults vary in size from 5/32 to 3/16 inch in length and about one-half that in width, and in colour from a pale green to a dark brown. They are strong-winged insects and overwinter as adults, presumably among the debris in fields and waste places. Egg-laying starts early in the season. The nymphs pass through five instars before the adult stage is reached. They are wingless, yellowish- or bluish-green, and in size and colour the earlier stages resemble young aphids but are readily distinguished from the latter by their ability to run about rapidly, by shorter and sturdier legs, and by harder bodies. In the later stages the developing wing pads are characteristic of the lygus bugs. In southern Alberta, Salt (6) reports two generations per season. No definite studies on this point were conducted in the White Fox area. However, in 1944 nymphs were rare in samples taken June 7 and 8 and adults were not numerous until about July 15. Even from July 31 to August 3, when a series of 21 fields was sampled, only 27% of the population was adult. As these data correspond closely to Salt's, and as few of the second generation matured at Lethbridge until September, it can reasonably be assumed that at White Fox the lower temperatures, which usually included at least a light frost by the middle of August, would result in few or no lygus bugs reaching maturity from the second generation.

The distribution of *Lygus* spp. attacking alfalfa is widespread. *L. oblineatus* Say is common in the eastern United States and Hughes (2)

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[4] The specific names in these genera follow the arrangement of Knight (3).

found it the most common species in northern Minnesota. Westward from Illinois, Iowa and Minnesota *hesperus* and *elisus* occur and Sorenson (9) and Carlson (1) in Utah, and Salt (6) in southern Alberta, record them as being common in alfalfa fields. In the White Fox area *hesperus* and *elisus* appeared to be the principal species.

HOST PLANTS AND NATURE OF DAMAGE

No extensive study of host plants was made but both *L. hesperus* and *elisus* are native to northern Saskatchewan and were found sparsely present whenever the native vegetation was sampled. They were found rarely in cultivated fields of oats, barley, flax and field peas in the White Fox district but counts of as high as 9.7 per sweep were recorded on an area heavily grown to lambsquarters, *Chenopodium album* L.

The two species are difficult to distinguish and at the time this study was made no differences had been established between the damage caused by each species. Thus they were grouped as *Lygus* spp. in the field data recorded. However, Stitt (11) has since shown that in California *L. hesperus*, when compared with *elisus*, can cause a slightly greater amount of flower fall and a highly significant increase in the number of non-viable brown seeds.

In all stages of development lygus bugs feed by sucking the juices from the host plant. Damage to alfalfa is chiefly to the growing parts and can be recognized through bud-blasting, flower fall and injury to both pods and seeds. Bud-blasting is the withering of the buds and the formation of so-called "white-tops"; flower fall is obvious in heavily infested fields, and injury to the developing seed results in the seed being brown in colour and more or less shrivelled. These symptoms are not always entirely due to lygus bugs and may be caused by factors such as climatic conditions, lack of fertilization, and injury from other insects such as thrips and the clover seed chalcid. However, Sorenson (7, 8, 9) and Stitt (10) have definitely related them to the prevalence of lygus bugs, and the large numbers of lygus bugs in the fields of alfalfa around White Fox leave no doubt as to their being the principal cause of seed damage as described later in this paper.

SURVEY OF BUG POPULATION

During each of the four years 1941 to 1944 a general survey of alfalfa fields in the White Fox area was made to obtain some idea of the general population of lygus bugs in that district. Table 1 presents a summary of the data based upon the average number of bugs taken by sampling with an insect net 14 inches in diameter. Each sweep was approximately 5 feet in length and taken with the man facing as nearly as possible into the wind and towards the sun. In recording data the numbers of adults and nymphs were not kept separate except in 1944 when, as previously stated, the samples contained an average of 27% adults. It was not always possible to sample at the same date period in each year. However, sampling was done at a time when serious damage could occur; that is, either when the crop was in full bloom and setting seed or after full bloom but before the pods had reached maturity.

TABLE 1.—AVERAGE FIELD POPULATIONS OF LYGUS BUGS IN DIFFERENT YEARS IN ALFALFA FIELDS IN THE WHITE FOX AREA

Year	Date	No. fields examined	No. of bugs per sweep*		Fields having 5.01–9.50 bugs per sweep	Fields having over 9.50 bugs per sweep
			Average	Range		
					%	%
1941	July 18–24	11	9.23	3.8–16.3	18	64
1942		33	0.45	—	—	—
1943	Aug. 15–18	27	6.17	0.8–18.4	30	26
1944	July 31–Aug. 3	21	13.52	2.5–50.0	21	54

* Total of adults and nymphs.

In California and Arizona, Stitt (10) found that a population of 5.01 to 9.50 lygus bugs per sweep resulted in a fall of 34.91% of the flowers that normally would form pods. For more than 9.50 bugs per sweep the value he obtained was 53.89%. If these relationships between lygus bug populations and flower fall hold in the White Fox area, then it is evident from Table 1 that losses in 1941, 1943 and 1944 were of serious proportions. As shown later in this paper, subsequent damage to developing seed undoubtedly also affects the seed yield of alfalfa. The reason for the light infestation in 1942 is not known. However, an exceptionally light snowfall during the winter of 1941-42, followed by a wet summer, may have caused a sharp reduction in the survival and propagation of lygus bugs.

EFFECT OF AGE OF STAND ON *Lygus* POPULATIONS

In 1943 a record was kept of the age of the stand of all 27 fields examined in the general survey summarized in Table 1; 13 of these fields were producing their first crop of seed, and 14 (which were producing the second, third or fourth crop) were grouped for the comparison with first crop stands.

The average number of lygus bugs per sweep was 3.6 (0.8 to 12.0) on the first crop stands and 8.5 (4.1 to 18.4) for the older stands. However, the 2 highest counts (12.0 and 6.6) in the first crop stands were on fields that had been seeded without a nurse crop in 1942 and had consequently made a heavy growth that year. The remaining 11 fields of first crop stands had been seeded with a nurse crop of cereals or flax and had not made nearly as vigorous a growth. As one might expect, the populations of lygus bugs increased more rapidly both in the pure stands of alfalfa in the fields seeded without a nurse crop and in the older fields than in the stands seeded with a nurse crop.

BROWN AND SHRIVELLED SEED AS RELATED TO LYGUS BUGS

The percentage of brown and shrivelled alfalfa seed has been shown by Sorenson (8) to be proportional to the lygus bug infestation, and Hughes (2) noted that badly shrivelled brown seed seemed of little value and that less than one-third of it germinated normally.

Since this type of seed is common in samples from northern Saskatchewan, 19 fields were sampled in 1944. The population of lygus bugs in each field had been estimated previously in the general survey summarized in Table 1. The seed samples were collected by walking through each field and picking ripe pods at random until a sample representative of the field had been gathered. Since frost damage is known to discolour alfalfa seed, all collections were made before early fall frosts had occurred. The samples were later threshed by hand and the seed separated so that even very small, shrivelled seeds were retained. The total number of seeds in each sample was counted and the "brown and shrivelled portion" recorded as a percentage of the total number.

The average proportion of brown and shrivelled seeds for the 19 fields sampled was 22% with a range from 9 to 51%. The average number of bugs per sweep for the same fields was 15 with a range from 2 to 50. When the two values from each field were correlated a correlation coefficient of + .58 was obtained. This value exceeds the 1% point.

A further study concerned the viability of different classes of seed. The economic value of these grades was measured by testing their viability at the Plant Products Laboratory at Saskatoon. As previously stated, the threshed seed was divided into a normal, and a brown and shrivelled, portion. This latter class was then subdivided by means of an air blast into heavy and light fractions. Table 2 presents a summary of the data obtained.

TABLE 2.—RELATIVE WEIGHTS AND PERCENTAGE GERMINATION OF NORMAL AND BROWN AND SHRIVELLED ALFALFA SEED

Class of seed	Percentage of original sample	Relative weight in percentage	Percentage germination, including hard seeds
	%	%	%
Normal	78	100	93
Brown and shrivelled			
Heavy fraction	7	90	26
Light fraction	15	48	3

The heavy fraction consisting of 7% of the total should correspond closely to the brown seed found in cleaned and graded samples undamaged by frost. The lighter fraction probably would be almost entirely blown over or screened out in normal threshing and cleaning processes.

From the data presented it is concluded that the brown and shrivelled alfalfa seed found in these samples was caused largely by lygus bugs and that its viability was low. The average of 22% of defective seed indicates a considerable reduction in yield and, since the range was from 9 to 51%, the loss was very high in individual fields.

CONTROL METHODS

The use of a pyrethrum concentrate and sulphur has been reported by Carlson (1) to have given increased yields on small plots. Sorenson (9)

considered the use of various insecticides insufficiently effective to be considered a practical means of control under field conditions. Hughes (2), however, found that pyrethrum dust had more knockdown than killing effect.

Leaving the second cutting of alfalfa for the seed crop has been suggested as a control by Sorenson (9) but this is impractical in northern Saskatchewan. Even when the first crop is allowed to set seed, serious damage from early fall frosts occurs in certain seasons. Thus any practice which delays the maturity of the crop increases this frost hazard.

Hughes (2) reported more or less control of *Lygus* spp. by certain cultural methods. Some of his data are briefly summarized as follows:

Untreated field	95.06 bugs per 50 sweeps
Thoroughly burned fields	23.62 bugs per 50 sweeps
Partially burned fields	67.97 bugs per 50 sweeps
Cultivated fields	65.00 bugs per 50 sweeps
Burned and cultivated fields	50.05 bugs per 50 sweeps

Carlson (1) has also reported an increased seed yield from a field where the dry grass was burned following the removal of the first hay crop. It has been a general custom in fairly recent years for certain alfalfa seed growers in the White Fox area to burn their fields early in the spring before little if any new growth appeared above the ground. No scientific basis was known to them for this procedure but it was generally believed that such fields gave an increased yield of seed. Burning in the fall and burning new stands were considered poor practices since both were often followed by winter-killing; burning late in the spring after the alfalfa was two or three inches high was also avoided since it delayed maturity. In most years the last two weeks of April was the best period to burn. It is not always possible to effect a complete burn, and unburned patches or strips often remain as a probable source of re-infestation. However, a heavy combine stubble and favourable weather may result in a fire that will sweep across a field in a very short time. The shorter stubble and lack of straw on fields harvested with a binder makes burning more difficult.

In 1943 a number of partially burned fields were observed and it was noted that areas of good seed-setting coincided with burned areas almost to a line, even though the edges often were very irregular. Following these observations a number of fields with patchy burns were studied in 1944. The samples for lygus bugs were based upon the average of 40 sweeps each from the burned and unburned parts of each field. Seed yields were secured from 10 paired samples of 1 square yard each. In all yield comparisons, samples were located not more than 2 rods apart and in many instances were within 10 feet of each other. The areas sampled in each field were subject to the criticism that differences, particularly in seed yield, may have been caused by the fertilizing effects of the ash residue or by favourable soil and moisture conditions which might have developed a heavier growth in the previous season and which consequently might be more likely to burn. It is not believed that either of these factors was effective. Certainly they could not account for differences in bug populations in adjacent areas. Observations on height and vegetative vigour indicated no difference in favour of the burned area. Soil type and moisture

appeared to be essentially the same and the amount of fertilizer per acre in in the ash from 1 to 1½ tons of vegetation is very small. Although spring burning is also a control for thrips, yet the numbers of thrips present seemed too small to greatly affect seed yield; definite studies on this point were not made.

A summary of the data obtained on the relationship between lygus populations and seed yield on burned and unburned areas in alfalfa fields in the White Fox area in 1944 is presented in Table 3.

TABLE 3.—LYGUS POPULATIONS AND SEED YIELDS ON BURNED AND UNBURNED AREAS IN ALFALFA FIELDS IN THE WHITE FOX AREA—1944

Field number	Average number of lygus bugs per sweep				Seed yields in pounds per acre			
	Date sampled	Burned	Unburned	Ratio of burned to unburned	Burned	Unburned	Difference	Percentage difference
1	June 7	0.5	0.3	1 : 0.6				
	June 28	3.3	8.7	1 : 2.6				
	July 5	12.0	20.3	1 : 1.7	86	36	50**	139
2	June 7	0.6	0.2	1 : 0.3				
	June 28	4.1	7.7	1 : 1.9				
	July 4	5.6	10.3	1 : 1.8	115	61	54**	89
3	June 8	0.4	0.2	1 : 0.5				
	June 28	2.8	4.9	1 : 1.8				
	July 18	7.6	8.5	1 : 1.1	127	74	53**	72
4	June 8	0.4	0.6	1 : 1.5				
	June 28	2.3	4.3	1 : 1.9				
	July 18	3.4	4.7	1 : 1.4	132	99	33	33
5	June 7	0.6	0.2	1 : 0.3				
	July 4	8.0	10.9	1 : 1.4	130	116	14	12
6	June 28	0.9	7.4	1 : 8.2				
	July 4	4.0	8.6	1 : 2.2	44	47	−3	−6
7	June 28	2.3	6.2	1 : 2.7				
	July 4	6.0	8.4	1 : 1.4				
8	July 4	4.1	7.6	1 : 1.8				
9	July 4	3.7	9.8	1 : 2.6				

** Exceeds the 1% point (odds in excess of 99 : 1).

Of the fields studied and listed in Table 3 there was no marked delay in the maturity of the alfalfa as a result of burning in any field except No. 6. This field was burned in May after the plants had made considerable growth. Consequently, it was set back 2 to 3 weeks in blossoming period and, had the fall season not been favourable, it might well have shown a greater reduction in seed yield because of frost damage. Fields No. 4 and 5 do not show a significant increase in seed yield. However, these fields were difficult to sample because the burning was irregular and the stands were patchy. In the remaining 3 fields it was easy to obtain comparable samples.

Of the 5 fields sampled on June 7 and 8 for lygus bugs, two, No. 1 and 3, showed only adult bugs. About 16% of the bugs in the other 3 fields were found to be nymphs. The total number of bugs present in any field at this time was small and did not consistently favour either the burned or the unburned areas. Evidently migration of over-wintered adults had occurred from the unburned areas after the alfalfa on the burned areas had developed sufficiently to provide food and shelter.

An altogether different picture had developed by the time later samples were taken from June 28 to July 18. The adults formed a very small percentage of the population, usually less than 5%. No counts were taken of the different instars among the nymphs but it was noticeable that the later stages were more numerous in samples from unburned areas. Thus it appears that a heavier and earlier deposition of eggs was made on the unburned patches as compared to adjacent burned stubble.

In order to interpret the data recorded on the differences in lygus populations between comparable burned and unburned areas, it is necessary to consider the situation at, and immediately following, burning. In April and early May, when the burn is effected, the alfalfa usually has made from 1 to 2 inches of growth which is entirely destroyed by the fire.

This growth may have already received some lygus eggs although no data on this point are available. If so, then the burning would have three main effects: (1) destruction of eggs, (2) destruction of adults trapped by the fire, and (3) destruction of all surface material so that neither food nor shelter would be available for a period of at least a few days. Normal egg-laying in the adjacent unburned patches would account for the more mature stages later found in the samples from these areas. Further, since food and shelter would not be present on the burned area, a concentration of adults might be expected in the unburned patches with a consequent heavier deposition of eggs, resulting in the higher numbers of nymphs recorded later in the season. As growth developed in the burned areas it is likely that there was an equalization of the numbers of the original adults, as well as of adults emerging from the nymphal stages, with respect to the two areas. This explanation agrees with the data and with observations made during the season; it also emphasizes the need for thorough burning.

As noted earlier in this discussion, the maturity of field No. 6 was delayed by late spring burning. In the other fields burning early in the spring did cause a slight initial set-back where growth had started. However, these latter fields had recovered completely by flowering time. Higher soil temperatures in the bare soil following burning, because of the greater absorption of the sun's rays, may account for the rapid recovery.

SUMMARY

1. Populations of *Lygus* spp. seriously affect seed yields of alfalfa in certain years in the White Fox area of Saskatchewan.

2. Fields producing the first seed crop tend to have lighter infestations of lygus bugs as compared to fields producing the second or later crop.

3. A significant positive correlation was obtained between the number of lygus bugs in a field and the percentage of brown and shrivelled seed taken before frost damage had occurred. This defective seed had a very low viability.

4. The reduction in lygus populations following burning of the stubble in early spring is thought to be effected by the destruction of hibernated adults, of their food and shelter, and of their eggs on the new growth of alfalfa.

5. Early spring burning of alfalfa fields is offered as a practical control measure for lygus bugs. Late spring burning should be avoided since it may seriously delay the maturity of the crop.

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THE POTATO-ROT NEMATODE, *DITYLENCHUS DESTRUCTOR* THORNE, 1945, ATTACKING POTATOES IN PRINCE EDWARD ISLAND[1]

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In 1931 potatoes coming from Prince Edward Island and New Brunswick were intercepted entering the United States. Injured tubers were submitted to Dr. G. Steiner, Division of Nematology, Washington, and the cause of the trouble found to be due to attack by the bulb or stem nematode, *Ditylenchus dipsaci* (Kuhn, 1857) Filipjev 1936. (1). This was the first time this nematode species had been found attacking the potato on the continent, although it had been recorded from this host plant in Europe.

On October 26, 1943, diseased potatoes were collected near Aberdeen Idaho, U.S.A. (2, 3). Nematodes recovered from these tubers were identified as *Ditylenchus dipsaci*. This was the first record of this nematode species attacking the potato in the United States.

In the past the "bulb or stem nematode" has been recorded from a large number of host plants and it has been evident for some time that a number of "strains" of this species existed. It was observed that populations of these different strains tended to have their own particular host range and it was suspected, by some workers, that several distinct species were involved.

The nematodes collected from Idaho potatoes were subjected to critical study by Mr. Gerald Thorne, Division of Nematology, U.S. Department of Agriculture, with the result that taxonomic characters were revealed which resulted in his naming and describing this form as a distinct species, *Ditylenchus destructor* (4). For this species Thorne suggests the common name of "potato-rot nematode". The diagnosis of *Ditylenchus dipsaci* was emended and referred to as the "teasel nematode". With further research Thorne hoped that it would be possible to definitely separate certain other forms of what was commonly known as the bulb or stem nematode.

In late November, 1945, during the course of inspection of potato stock in Prince Edward Island, Mr. G. C. Ramsay, Seed Potato Inspector, Division of Plant Protection, observed potato tubers which appeared to show some of the symptoms of bacterial ring rot. Specimens were collected and turned over to Mr. S. G. Peppin, District Inspector-in-charge, Mr. F. M. Cannon, Division of Entomology, and Mr. R. R. Hurst, Division of Plant Pathology (all located at Charlottetown). After examination of the tubers it was evident to these officers that the injury was not due to insects, fungi, or bacteria. Nematodes were observed in the tubers and it was suspected that they might be responsible for the diseased condition of the potatoes. Accordingly, the two latter officers forwarded samples of these tubers to Ottawa for more definite determination of the cause of the trouble. These potatoes were recovered from a farm near York which lies

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about five miles northeast of Charlottetown. The infected stock was Green Mountain and it is reported that, as far as could be ascertained at the time, the trouble was apparently confined to a certain small, low area in one field.

Examination of the nematodes recovered from these Prince Edward Island potatoes revealed that they represented a species of *Ditylenchus* and that they were very evidently responsible for the injury to the tubers. Critical study of the characters of this nematode species showed that they corresponded closely with those described for *Ditylenchus destructor* by Thorne. Furthermore, the characters described by Thorne in his emended description of *Ditylenchus dipsaci* (teasel nematode) did not agree with those observed on the nematodes recovered from the P.E.I. potatoes. However, as Thorne was still giving attention to the taxonomy of the "bulb or stem nematode", and had an expert's knowledge of this problem, specimens were submitted to him for study. Mr. Thorne reported that the nematode found attacking the potato in Prince Edward Island was the potato-rot nematode, *Ditylenchus destructor* Thorne, 1945.

In his article dealing with *Ditylenchus destructor* (4), Thorne states that he compared the characters of this species "with specimens collected from potatoes intercepted by inspectors in ship's stores at the port of Boston in 1932 and found them to be the same". While it is rather probable that these potatoes came from Prince Edward Island, Thorne was careful to refrain from making this statement until more definite evidence had been secured. However, with the recovery of specimens this year we now have clear evidence that the potato-rot nematode is present in a small portion of one field in Prince Edward Island.

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THE DIAGNOSIS OF BACTERIAL BLACK CHAFF OF WHEAT[1]

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INTRODUCTION

Divergent views on varietal resistance to bacterial black chaff of wheat have been held by various investigators and it appears likely that some, at least, of the conflicting statements that have appeared have been due to a failure to distinguish between bacterial black chaff and certain other diseases. For this reason it seems desirable that the means of diagnosing bacterial black chaff be given special attention.

For a good description of bacterial black chaff and an accurate, though limited, description of its causal organism, we are indebted to several investigators who studied the disease shortly after its appearance in severe epidemic form in Kansas in 1915. Although Melchers (18) had begun to study it two years earlier, the 1915 epidemic resulted in increased activity by him and by others in an endeavour to determine the characteristics of the disease. For example, Smith (21) stated that, in the summer of 1917, 14 persons were working under his direction on this problem. Following publication of the description of the disease (20) it soon became apparent that it was widespread in the cereal-growing areas of the world and was capable of occurring in epidemic form whenever conditions favourable to its development were present (5, 6, 8, 12, 19, 24). In Manitoba, it was severe in 1928 (16), and again in 1935.

Some years ago, owing to inadequate diagnoses, dark discolorations of wheat plants were generally attributed to bacterial black chaff. Subsequently, however, when wheats of widely divergent genetic origin were being used as parents in intensive plant breeding programs, it soon became evident that discolorations of the black chaff type were not all of identical origin (9). Some of the discolorations did not appear to be of bacterial origin. In fact, it soon was shown that dark discolorations, which could easily be confused with bacterial black chaff, might occur either alone or associated with that disease (10). Furthermore, it is now known that macroscopically indistinguishable discolorations of more than one origin may be found on a single head of wheat. Consequently, in making field plot estimates of bacterial black chaff severity, it is necessary to use suitable diagnostic methods in arriving at a correct assessment of the intensity of the disease.

Because of the discolorations of mixed origin just referred to, efforts have been directed by the writer toward the development of diagnostic methods that would facilitate the making of determinations from relatively large numbers of lesions. It was hoped that the serological method developed in Russia (2), and mentioned below, might be adaptable for use on the bacteria obtained directly, that is, without pure culturing, from individual lesions of bacterial black chaff. Unfortunately, in the presence of

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plant tissues and juices, this method did not prove reliable. With this as with other methods, it was found necessary first to isolate the organism. The purpose of the present paper is to outline the use of various aids to the diagnosis of bacterial black chaff as practised at this laboratory.

PROCEDURE FOR THE DETECTION OF BACTERIAL BLACK CHAFF

Examination under Low Magnification

To determine whether or not the disease is present, discoloured heads, stems, and leaves of wheat are examined with the aid of a dissecting microscope, or a hand lens, for the presence of bacterial exudate, which may be present either in the form of a thin transparent scale or as yellowish beads. When this examination is made in the laboratory, the heads are illuminated by a strong beam of light, preferably passed through a pale blue or green filter. Such an examination will reveal also the pycnidia of *Septoria* spp. (25), if present, or any dark spore masses of pathogenic or saprophytic fungi. The absence of pycnidia should not be regarded as proof that Septoria glume blotch is not present; neither should, in itself, the presence of spore bodies or masses be accepted as evidence that bacterial black chaff is not present, for saprophytic or weakly parasitic fungi sometimes fructify on tissues parasitized by the bacterial black chaff organism.

In making a visual examination, it should be borne in mind that both microbiological agents and environmental factors may play a part in giving rise to visually similar discolorations. Generally, however, certain symptoms characterize each of the discoloration types. In bacterial black chaff (20), the glume lesions consist typically of dark-brown to black, more or less sunken stripes, occurring chiefly on the upper parts of the glumes, yet heavy infections sometimes have a chocolate-brown appearance, markedly resembling the discolorations caused by *Septoria nodorum* Berk. In Septoria glume blotch (25), the chocolate-brown to black spots often bear punctiform, black pycnidia, especially on the exposed portions of the lemma, but when these are not present further diagnostic study is necessary. In basal glume rot (15), the discoloured areas occur chiefly at the base of the spikelet, or radiate out from the base. In brown necrosis (13, 17), and infections by *Helminthosporium sativum* P. K. & B. (23), the discolorations on the heads are often very similar to those of bacterial black chaff. Discolorations of this type, which occur in nature, should be suspected of being bacterial black chaff until isolation tests have proved negative for the bacterial black chaff organism. In pseudo black chaff (3), the blackening is especially intense and occurs mostly on the apical portions of the glumes. In Alternaria blotch (10, 13), the discolorations are usually more diffuse than in bacterial black chaff and often occur opposite unextruded anthers.

A diagnostic feature that is helpful when classifying the discoloration being studied is the location of the majority of the discoloured areas. Bacterial black chaff, Septoria glume blotch, and brown necrosis occur primarily on the outer glumes and exposed apices of the lemmas, while basal glume rot and Alternaria blotch occur chiefly on the lemmas and, to a lesser degree, on the outer glumes.

One point regarding the symptoms of bacterial black chaff which does not seem to have been mentioned in the literature, is that, in varieties of durum wheat (*Triticum durum* Desf.), the glume discoloration is light brown and diffuse rather than dark brown and sharply delimited as in common wheats (*T. vulgare* Vill. = *T. aestivum* L.).

The kernels are shrivelled and may be darkened irregularly following a severe attack of bacterial black chaff (Figure 1). Black tip, due to infection by the basal glume rot organism, differs from bacterial black chaff discoloration in being confined to the embryo end of the kernel.

Dark discolorations of the rachides and of the upper internodes of the culm occur in brown necrosis as well as in bacterial black chaff. Similar discolorations appear to result from purely physiologic causes (10, 14). When they do, the disorder may be referred to as internodal melanism (10). In bacterial black chaff, internodal discolorations are found only when the attack is severe, but in brown necrosis they appear to be a common feature of the attack. On the other hand, dark discoloration of the peduncles is a common feature of bacterial black chaff, but similar discoloration may also be present in plants affected with Septoria glume blotch, Alternaria blotch, or brown necrosis.

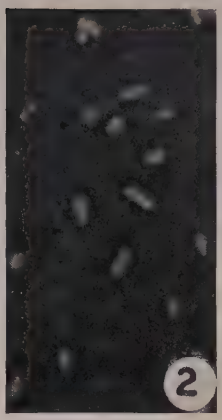
The foliage symptoms of bacterial black chaff are as described by Melchers (18), who mentioned glistening varnish-like areas with lesions denoting bacterial infection. Such semi-translucent areas are characteristically present in the leaf lesions. Histological examination has shown that the translucency is due to the partial dissolution of the mesophyll and the occlusion of all air spaces by bacterial masses. Both weather conditions and varietal differences in the host appear to influence the extent of the translucency. Unfortunately, bacterial infection of the leaves cannot be accepted alone as evidence of the presence of bacterial black chaff. Similar symptoms may be caused by the basal glume rot organism, *Pseudomonas atrofaciens* (McC.) Stev. Several heavy leaf infections, both in spring and winter wheats, have proved, on isolation of the causal organism, to have been caused by *Ps. atrofaciens*.

As a rule, the determination of bacterial black chaff by examination under low magnification, alone, can be relied upon only if bacterial leaf lesions are found in association with the typical head and neck lesions, or if a bacterial exudate is associated with the typical head and neck lesions.

Examination under High Magnification

According to Fröier (6), J. Lindberg, at Svalöf, Sweden, established the intracellular and intercellular nature of the bacterial black chaff disease as long ago as 1918. Bamberg (1), similarly, observed bacteria both in the cells and in the intercellular spaces, and his observations are in agreement with those of the writer.

In the examination of tissues suspected of being infected with bacterial black chaff, an attempt should be made to prepare a bacterial smear. Either positive or negative staining is effective, but the negative staining method described by Burkholder (4) is, perhaps, the most rapid. With this method, the smear is made in Congo Red stain and acidified with acid



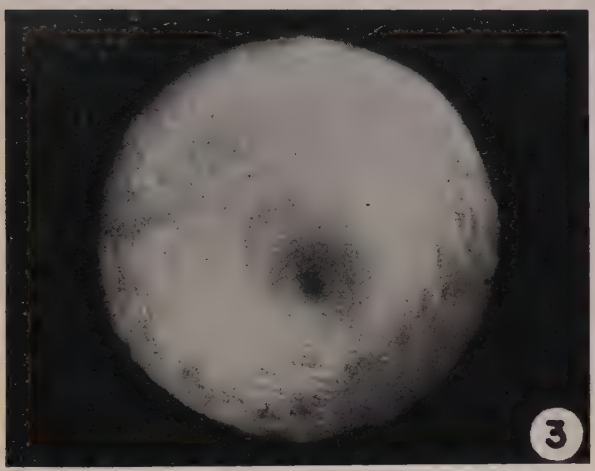


FIGURE 1. At left, 12 kernels from a head of Thatcher wheat that was severely attacked by bacterial black chaff. At right, 12 kernels from a healthy head. ×2.

FIGURE 2. Photomicrograph of a Congo Red smear preparation made from a lesioned glume of wheat. ×1500.

FIGURE 3. A colony of *Xanthomonas translucens* growing on beef peptone agar, photographed by oblique transmitted light. ×10.

FIGURE 4. The drop agglutination test. At left, agglutination following suspension of *Xanthomonas translucens* in the immune serum diluted to 1 part in 39 of phenolated saline. At right, no agglutination following suspension of *X. translucens* in phenolated saline. If a species of bacteria other than *X. translucens* had been suspended both drops would have the appearance of the drop on the right. Note that the few clumps remaining in the saline suspension due to incomplete dispersion of the bacterial mass are readily distinguishable from true agglutination. True agglutination, as shown in the drop at left, results in the clearing of the liquid. ×2½.

alcohol before examination at a magnification of about 900 diameters. Smears thus prepared from leaf or stem lesions of bacterial black chaff always show bacteria in great numbers, the bacteria appearing as white rods on a blue background; in glume lesions, the bacteria are sometimes scarce, although usually numerous (Figure 2).

The presence of bacterial rods in large numbers is not sufficient evidence that the disease is bacterial black chaff. The bacteria may indicate the presence of the basal glume rot organism or of a bacterial saprophyte. In a large percentage of samples, cultural studies also are required.

Isolation

For purposes of isolation, a small piece of lesioned tissue, usually from 3 to 10 sq. mm. in area, is dipped momentarily in 70% alcohol, then immersed for a period of $\frac{1}{2}$ to 1 minute in 0.1% mercuric chloride solution. After diffusion in sterile distilled water has been permitted for not less than 5 minutes, the piece is torn apart, aseptically, and 4 dilution plates of beef peptone agar are poured from it. The plates are held for 10 days at 26° C. in an inverted position. Repeated isolation attempts on the same samples have shown that this method is effective on the first attempt in over 90% of the samples of bacterial black chaff.

Appearance of Colonies of *Xanthomonas translucens* (J. J. & R.) Dowson *emend.* Hagb.

The colonies in the isolation plates are viewed with a hand lens in oblique transmitted light (Figure 3). The well-developed, yellow, surface colonies of the bacterial black chaff organism and of all other special forms of the species, are characterized by minute, interblending, internal striations (22). These striations are present only when the colonies are sown thinly. Godkin (7) stated that they appear in f. sp. *hordei* Hagborg in 12 days, and in f. sp. *undulosa* (S. J. & R.) Hagborg in 9 days, but the writer has found that development of the striations depends less on special form differences than on the distance between colonies. Even in cultures from barley, the striations develop in less than a week if the colonies are widely separated.

On the tenth day of incubation, transfers are made from the isolation plates. These isolates are tested for pathogenicity as soon as they are sufficiently well developed.

Tests of Pathogenicity

The pathogenicity of each isolate is tested at about 25° C. by the inoculation of from 10 to 15 leaves of wheat, oats, barley, and rye seedlings. For uniformity, the same cereal varieties are used from year to year, viz.: Thatcher wheat, Victory oats, Star barley, and Prolific (Spring) rye. In this inoculation, a nichrome needle is flamed and allowed to cool, then dipped in the inoculum and used to wound the very young primary leaves. After being held at about 25° C. for 7 to 10 days under good light conditions and preferably at a relative humidity of about 75%, the inoculated plants are examined for infection. *Xanthomonas translucens* f. sp. *undulosa*

causes water-soaked areas, which later turn a translucent brown on wheat, barley, and rye; *X. translucens* f. sp. *cerealis* causes similar infections on these hosts and also on oats. However, for practical diagnostic purposes, both of these may be regarded as the bacterial black chaff organism, as under field conditions neither of them appears capable of attacking oats.

The pathogenicity test is quite effective in revealing the bacterial black chaff organism and has the additional advantage that with it *Ps. atrofaciens*, the basal glume rot organism, produces a distinctive reaction. This organism causes the wound margins on all four hosts to turn dark brown to black.

Serological Identification

When the results of diagnoses are required at the earliest possible moment, serological methods are particularly suitable. Certain Russian workers (2, 8) have pointed out the practical advantages to be gained in replacing time-consuming morphological and biochemical methods of identification with a rapid drop-agglutination test. They have found this method reliable in distinguishing the bacterial black chaff organism from other bacteria associated with wheat. In their method, a drop of control serum and also a drop of the specific agglutinating serum are placed on a microscope slide. A mass of the bacteria, the size of a pin head, is transferred to each drop, mixed well, and allowed to remain for a few minutes at room temperatures. With positive results, the specific serum becomes cleared with the appearance of granular, whitish aggregates, and the control serum remains unchanged; but with negative results, both drops retain their homogeneous, greyish appearance.

The writer has confirmed, with numerous isolates, the usefulness of the drop-agglutination method as a rapid test for identifying the bacterial black chaff organism in pure culture. In place of the control serum, he has used a phenolated physiological salt solution with equally satisfactory results (Figure 4). The drop-agglutination method furnishes immediate results and for this reason is especially advantageous in certain cases, but with it, as with the pathogenicity test, isolation is a prerequisite. Both methods are well adapted to the testing of large numbers of isolates, but the drop agglutination test fails to differentiate between the special forms of *X. translucens*. To thus differentiate them is the special merit of the pathogenicity test.

Cultural Identification of the Species

Cultural identification of the species *X. translucens* is done after the non-pathogens or serologically non-reactive isolates have been eliminated. In the writer's experience, all isolates not eliminated by either of the other tests have proved to be true to the remaining determinative characters of this species, described elsewhere (11). Before making the cultural studies, a purity test should be made by the examination of well-separated colonies in dilution plates. If necessary, the organism may be readily re-isolated in pure culture from a seedling leaf infected in the pathogenicity test. The re-isolate also is tested for purity and for pathogenicity or agglutination before being used in the remaining determinative tests.

DISCUSSION

Dark discolorations on the heads and stems of wheat plants often cause concern when they appear either in experimental plots of new wheat varieties or in the commercial wheat crop. As such discolorations may result from several different causes (3, 9, 10, 13, 14, 17) the use of suitable diagnostic methods is prerequisite to the subsequent employment of control measures. Where the attempted control measure consists of breeding for resistance to any of the diseases that cause dark discolorations, diagnostic tests are a necessary supplement to field studies on wheats of hybrid origin.

There has been some confusion in the past in respect to the nomenclature of diseases resembling and including bacterial black chaff. The designation "black chaff" was originally made by Melchers (18) and used by several authors (1, 5, 18, 22, 24) with reference to the bacterial disease caused by the organism now known as *Xanthomonas translucens* f. sp. *undulosa*. Several investigators have used it also to embrace dark discolorations that in the light of more recent studies were probably not bacterial black chaff. In addition, the combination "so-called black chaff" has been used in the literature as if synonymous with brown necrosis (17). It is now believed that, with proper emphasis on careful diagnosis, and with the adoption of the correct distinctive names for the various discoloration diseases—Septoria glume blotch, bacterial black chaff, brown necrosis, Alternaria blotch, pseudo-black chaff, and internodal melanism—confusion can be largely avoided.

It is not always difficult to diagnose bacterial black chaff. Where the symptoms are sufficiently well developed, isolation and identification of the causal organism may be omitted. This is particularly the case with a variety such as Thatcher, which is relatively resistant to brown necrosis and Alternaria blotch. On the other hand, in experimental plots where numerous strains or varieties of wheat are present, discolorations of various kinds are apt to occur. As it would be impractical to determine the cause in every individual lesion, resort may be had to a complete diagnosis on representative sample lesions.

Aside from its use in the detection of bacterial black chaff in breeding plots, an adequate diagnosis can serve a useful purpose as a preliminary step in the selection of control measures for diseases in the commercial crop. Any severe outbreaks of dark discolorations may be tested for the presence of bacterial black chaff. If other causal organisms are involved, the proportion attributable to bacterial black chaff may be assessed in order to determine the advisability of adopting measures to prevent its recurrence.

SUMMARY

Because of the difficulty experienced by many investigators in diagnosing bacterial black chaff of wheat, a procedure of diagnosis that has proved effective is presented. All the essential parts of this procedure can be performed with the aid of equipment ordinarily available in a plant pathological laboratory. The procedure consists of the examination of macroscopic and microscopic portions of the diseased tissues, the isolation of the causal organism, and the identification of it by either a study of its pathogenic or of its serologic reactions, coupled with a study of its morphology and physiologic properties.

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ERRATUM

In the January 1946 issue of *Scientific Agriculture* (Vol. 26, No. 1) amend the formula on page 30 to read:

$$\frac{\text{Sum of all numerical ratings}}{\text{Total number of seedlings} \times \text{maximum rate}} \times 100 = \text{Disease rate}$$